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MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

THE INSECTS ATTACKING STORED WHEAT IN THE PUNJAB, AND THE METHODS OF COMBATING THEM, INCLUDING A CHAPTER ON THE CHEMISTRY OF RESPIRATION

BY

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PREFACE.

THE subject of this Memoir was first taken up in 1911 with the object of providing a cheap and efficient method of preserving seed wheat in small stores against damage by "weevils." These stores were to be small and cheap enough to be suitable for the larger landowners or large enough for village communities. They were also required to be sufficiently simple in construction and in method of working to be suitable for the ordinary Punjab village and its inhabitants.

The preservation of the grain while mainly undertaken with the object of securing sound seed wheat must also leave it fit for human consumption since the needs of the owner might at any time drive him to sell his grain.

These restrictions narrow the possibilities very considerably and put out of court practically all the insecticides and chemical deterrents known.

I therefore turned to the use of carbon dioxide and cheap bins of mud, lined with galvanised iron sheeting to protect the contents against rats or other vermin likely to gain access to an ordinary building of mud or brick, and with the joints soldered to render them gas-tight so that the bin could be filled with the gas carbon dioxide to asphyxiate the insects present in the grain and in the bin.

Bins fulfilling these conditions were erected on the Lyallpur Farm and the experiments described in Chapter II made. These experiments include a series of necessary subsidiary investigations to obtain the required information on conditions of germination, the effect of the gas carbon dioxide, and the moisture contents of wheat as it is found in the Punjab markets, etc. Before the end

of 1912 the variations in the results showed that the entomological information available was insufficient and that we were working in the dark as to the types of insects causing the damage. The entomological staff at the Agricultural College, Lyallpur, was unable to cope with the problem and I consequently asked the then Agricultural Adviser to the Government of India (Mr. J. Mackenna) in March **1913** to post Mr. A. J. Grove, Supernumerary Entomologist, to Lyallpur to take up this side of the work. The matter was officially represented by the Punjab Government in **1913**, and early in 1914 Mr. Grove joined the Punjab Agricultural Department on deputation. By this time the problem had broadened itself to include the storing of wheat in large granaries or elevators, and when Mr. Grove arrived our principal object was to secure information which would enable us to deal satisfactorily with grain in large bulk.

On joining at Lyallpur Mr. Grove at once took up the study of the life-histories of the three insects which his tours in the two preceding years had shown to be the ones causing most damage in stored wheat in the Province, and simultaneously with this I extended my investigations on the effect of carbon dioxide and other gases on specimens of the insects isolated by him.

These experiments are described in Chapters III and IV, and Mr. Grove's work on the life-histories of the insects in Chapter I and " the effect of moist and dry atmospheres " in Chapter V.

The division of the work has left Mr. Grove responsible for all the entomological investigations mentioned in the Memoir and included in Chapters I and V and the naphthalene experiments at Gurdaspur mentioned in Chapter VI, all of which are by his pen.

The chemical and physical studies mentioned elsewhere in the note and the mechanical remedial measures are the work of myself, my assistant Bh. Jagat Singh, M.SC., Assistant Professor of Chemistry in the Lyallpur Agricultural College, and other members of the chemical staff. The entire work has been done in the closest collaboration and all recommendations are made jointly.

The Memoir is a report to the Punjab Government on the "wheat weevil" problem. It has been carried out (for the most part) in the Government laboratories at Lyallpur, and I wish to gratefully acknowledge the Government grants without which the work herein recorded could not have been done. These grants were included in the budget of the Agricultural College, Lyallpur, for the years 1911-1914.

J. H. BARNES.

LYALLPUR,
July, 1915.

Mr. A. J. Grove having joined the Mesopotamia field force, he has been unable to correct the second proof of that portion of the Memoir for which he is responsible (*see* above), and Mr. T. Bainbrigge Fletcher, Imperial Entomologist, has very kindly read the final proofs of the entomological chapters for us.

J. H. BARNES.

October, 1916.

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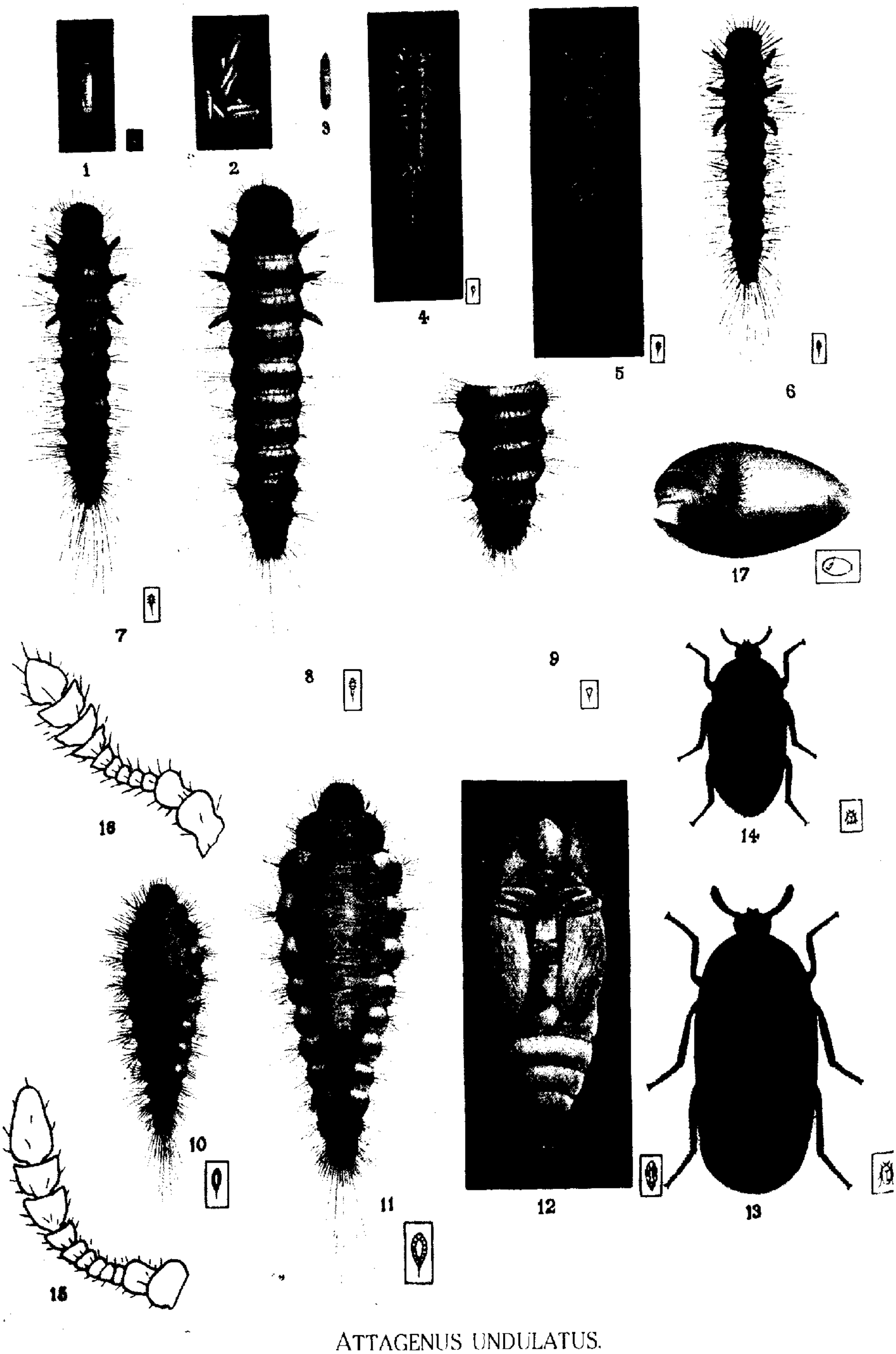

ATTAGENUS UNDULATUS.

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CHAPTER I.

ENTOMOLOGICAL.

THE study of the insects which damage stored products, and particularly wheat, has not hitherto received from economic entomologists in India the attention merited by the amount of damage and loss for which such insects are responsible. This chapter contains a record of the observations carried out upon the insects found in stored wheat in the Punjab. The work was carried out at Lyallpur, so that the results obtained will refer more especially to that place, and although these results would be applicable to most places in the Punjab, yet the investigations show that in all probability the insects would behave rather differently in other parts of the Province. This variation, as will be shown later will refer mainly to differences of climatic conditions and more especially to humidity.

THE INSECTS WHICH ARE FOUND IN STORED WHEAT IN THE PUNJAB.

A systematic examination of wheat stores in the Province revealed the following insects as occurring commonly :—

Coleoptera.
Læmophlœus sp. (Cucujidæ).
Attagenus undulatus, Motsch. (Dermestidæ).
Rhizopertha dominica, Fb. (Bostrychidæ).
Tribolium castaneum, Hbst. (Tenebrionidæ).
Latheticus oryzæ, Waterh. (Tenebrionidæ).
Alphitobius piceus, Ol. (Tenebrionidæ).
Calandra oryzæ, Linn. (Curculionidæ).
Calandra granaria, Linn.[1] (Curculionidæ).
Lepidoptera.
Sitotroga cerealella, Oliv.

From this list it will be seen that the insects are mainly beetles. All of them are small, and to the untrained eye so very much alike, that the native grain dealers and zemindars are unable to separate them from one another, with one or two exceptions, in which cases vernacular names have been given to the insects. For instance the larvæ of *A. undulatus* which are different from those of most other insects found in stored wheat have been named *khapra*, but when they were shown the adult beetle the grain dealers could not distinguish it from other beetles such as *C. oryzæ*, *R. dominica* and *T. castaneum*, all of which are given the name *susri*. In fact the zemindars and grain merchants recognize only two kinds of insects attacking wheat, *viz.*, *khapra* and *susri*. In one or two cases, however, it was found that two kinds of *susri* were distinguished, *C. oryzæ* being differentiated from the other beetles and designated the *sund wali susri* (the insect with a trunk like an elephant) from the snout resembling an elephant's trunk.

The next problem to be solved was to determine which of these insects were of importance.

Læmophlœus sp. In examining wheat stores this insect was only found in quantity when the wheat had been badly attacked by other insects. Its occurrence was not so common as that of the other beetles, and from general observations it did not seem to be of any great importance. Experiments in the laboratory on the lines carried out with *T. castaneum* recorded later

[1] This insect was only found in two localities and is much rarer than *C. oryzæ*.

showed that this insect does not attack sound grain but lives on grains damaged by other insects or in the floury frass produced by them. It is nearly always found in conjunction with *T. castaneum.*

Attagenus undulatus. Practically no information regarding this insect is available. One author[1] merely records its occurrence and another[2] suggests that in all probability this insect lives on other insects. Investigation showed, however, that in the larval stage the insect attacks wheat grains (*see* p. 182). It is found practically everywhere in the Punjab, and is in fact an important pest of stored wheat in this province.

Rhizopertha dominica. This is a fairly well-known insect, and it attacks and damages wheat in both the larval and adult stages. It is found in most places in the Punjab.

Tribolium castaneum. This insect which Lefroy has called the Red grain beetle, occurs practically wherever wheat is stored, but, as the following experiment shows, it is not really an active agent in damaging sound grain. On July 23, 1914, four small glass dishes were taken, containing the following :—

(*a*) Whole grains of wheat.

(*b*) Grains of wheat which had been damaged by other insects, but in which there were no insects present.

(*c*) Grains of wheat, some of which had had a portion cut off from the embryo end, some from the apex and some split longitudinally.

(*d*) Ground wheat.

Into each of these, 20 active beetles were placed. It had been previously determined that *T. castaneum* would breed well in ground wheat so that (*d*) would act as a control to the other three. The insects were left undisturbed until August 27th, when it was found that in—

(*a*) all the beetles were dead ; the grain had been attacked a little where the pericarp was broken and no live larvæ were present. One or two eggs had been noticed but the larvæ from them had died ;

(*b*) 14 beetles were alive, 5 dead ; eggs and larvæ were present and much frass had been produced, the damaged grain having been practically eaten away ;

(*c*) 6 beetles were alive, 13 dead, and several dead larvæ were present : much frass had been produced, and all the cut surfaces had been attacked almost equally. The larvæ had evidently passed through several moults as many cast skins were found, but they had evidently died eventually ;

[1] Cotes, E. C. Notes on *A. undulatus. Indian Museum Notes.* Vol. III, 1894, pp. 23 and 119.

[2] Lefroy, H. M. *Indian Insect Pests* (1906).
,, ,, *Indian Insect Life* (1909).

(*d*) 55 beetles were alive, 2 dead, and a large number of larvæ. The surface of the wheat was covered with cast larval skins.

From this experiment it will be seen that the ideal food for *T. castaneum* is ground wheat or flour ; that it cannot live on whole grains of wheat, but can live on grains which have been damaged by other insects or mechanically ; and in fact it may be said that this insect is not a grain beetle but a flour beetle.

Latheticus oryzæ. There is not much information available regarding the occurrence of this insect in India. Lefroy[1] (1909) does not mention it, and Fletcher[2] (1914) merely records it in a list of insects attacking stored products. It is very closely allied to *T. castaneum* and very similar to it, and this may account for its not having been recorded. It does not occur as commonly as *T. castaneum* but has been found at Lyallpur, Ludhiana, Jagraon, Sargodha, Sangla Hill and Jaranwala. The insect was tested in exactly the same way as *T. castaneum* with the same results.

Alphitobius piceus. This beetle was only found in wheat which had become damp or which had been badly damaged. It was nearly always found associated with attack by white ants. It is a large black beetle and the larva is a whitish yellow grub with a darker coloured head. All attempts to rear it on wheat in any form failed ; probably it is merely a scavenger and does not attack the grain.

Calandra oryzæ. This insect is perhaps the best known of all the insects attacking stored wheat in India and is a pest of great importance, the insect attacking the wheat in both larval and adult stages. It is found in most parts of the Province.

Calandra granaria. Little is known of the occurrence of this insect in India. Lefroy[1] (1909) and Fletcher[2] (1914) mention it, but no account of it is given. The writer (A. J. G.) has recorded it in two places : Rewari (2 specimens), and Gurdaspur (a few specimens mixed with *C. oryzæ*) ; but it is not nearly as common as *C. oryzæ* nor of such importance.

Sitotroga cerealella. This small grain moth was only found in any numbers in the Gurdaspur district, and though it probably occurs in other places, it was not observed, and the grain dealers were apparently not familiar with it, so that except in the Gurdaspur district it is probably not of much importance. The larvæ damage the grain (*see* pp. 203 and 204).

It will be seen, therefore, from what has been recorded above, that only three of the insects found in the stored wheat are actively responsible for damage to the wheat, *viz.*, *A. undulatus*, *R. dominica* and *C. oryzæ*.

[1] *Loc. cit.*

[2] Fletcher, T. Bainbrigge. *South Indian Insects*, 1914, Government Press, Madras.

DISTRIBUTION.

Having determined which insects are responsible for the destruction of the wheat, the next point to be considered is the distribution of the various insects in order to throw some light upon their relative importance in the various places in the Punjab. In August and September 1913 a short tour was made to some of the chief wheat centres in the province, Ludhiana, Moga, Ferozepur and Lyallpur being visited. At this time very little was known of the insects attacking wheat in the province with the possible exception of *C. oryzæ*, and the visit was of the nature of a preliminary examination to determine what insects were to be found in the wheat. In August 1914 a more comprehensive inspection of the chief markets of the province was made, and in addition to the examination of the wheat stores in the market or *mandi* itself, wherever possible, one or two of the villages in the vicinity from which the *mandi* received its supplies, were also examined. The results of these observations have been tabulated below :—

TABLE I.

Name of place	*A. undulatus*	*R. dominica*	*C. oryzæ*	REMARKS
LYALLPUR.				
Lyallpur Mandi ...	ccc*	cc	c	Average rainfall 12·66 inches. Subsoil water 50—80 feet below the surface. Cultivation only possible where canal irrigation water available. Atmosphere generally speaking dry, but irrigation has raised the humidity.
Chak No. 65 ...	cc	cc	f	
Kamalpur ...	c	c	...	
Chak No 36 ...	c	c	...	
Panjwar ...	...	c	...	
Chukera ...	...	c	...	
GURDASPUR.				
Gurdaspur Farm ...	...	...	ccc	Average rainfall 34·02 inches. Subsoil water very near surface. Conditions moist.
Nawanpind ...	...	c	c	
Bābi	...	f	f	
Mustafabad ...	f	...	c	

* *N.B.*—In the table the following signs have been used :—

ccc indicates that the insect is very common.
cc ditto common.
c ditto fairly common.
f ditto found, but in small numbers.

TABLE I.—*continued.*

Name of place	*A. undulatus*	*R. dominica*	*C oryzæ*	REMARKS
AMRITSAR.				
Amritsar Mandi ...	cc*	c	c	Average rainfall 23·98 inches. Subsoil water 10 to 20 feet below surface. Moist.
Sultanwind ...	...	...	ccc	
Kot Said Mohd ...	cc	f	f	
JULLUNDUR.				
Jullundur Mandi ..	cc	f	c	Average rainfall 26·8 inches.
Phagwara Mandi...	c	c	cc	
LUDHIANA.				
Ludhiana Mandi...	c	...	c	Average rainfall 26·23 inches. Subsoil water 20 to 30 feet below surface. Irrigation mainly from wells.
Chapki ...	...	...	c	
Jagraon Mandi ...	c	c	c	
Moga ...	cc	f	...	
SIRSA.				
Sirsa Mandi ..	ccc	...	...	Rainfall about 16 inches. Conditions very dry.
Buraguda ...	cc	...	...	
Khaipur ...	ccc	...	...	
REWARI.				
Rewari Mandi ...	f	...	f	Rainfall about 25 inches. Conditions dry.
Rampura ...	f	...	...	
Gokalgarh ...	c	...	...	

* *N.B.*—In the table the following signs have been used :—

ccc	indicates that the insect is very common.	
cc	ditto	common.
c	ditto	fairly common.
f	ditto	found, but in small numbers.

From the table, it will be seen that although generally speaking all three insects are found all over the Punjab, yet in some districts they are not of equal importance, whereas in others all three seem to be of almost equal importance. For instance, in districts such as Lyallpur, Moga, and Sirsa *A. undulatus* seems to be the commonest insect, whereas in the Amritsar and Gurdaspur districts *C. oryzæ* is the greatest source of loss. *R. dominica* seems to be generally troublesome. In the districts of Jullundur, Ludhiana, Jagraon, and Phagwara all three appear to be of almost equal importance.

In considering these results it must always be remembered that they were obtained upon a single visit and examination, but in each case the actual observations were supplemented by information obtained from grain dealers and merchants living in the place, many of whom had had lengthy experience of stored grain. The results are also supported by experiments connected with the effect of dryness and moistness on these three insects, but it is not proposed to discuss this point here as it will be dealt with in another place (see p. 261 *et seq.*).

In order further to supplement these results samples of insects found in wheat were obtained from a number of other places in the Punjab. This was done through the kindness of Mr. P. MacBrien of Messrs. Sanday Patrick & Co.; Mr. A. J. Peake of Messrs. Clements Robson & Co.; and Mr. Fritz Gidion of Messrs. Louis Dreyfus & Co., the representatives of these wheat exporting firms at Lyallpur. Tubes were sent to the sub-agencies of these firms in different places and the insects obtained placed in them and returned to Lyallpur for identification.

The record in tabular form is as follows :—

TABLE II.

Name of place	Collector	*A. undulatus*	*R. dominica*	*C. oryzæ*
Lyallpur	L. D.*	+	+	+
	S. P.	+	+	+
Chak Jhumra ...	C. R.	+	+	−
Sangla Hill ...	S. P.	+	+	−
	C. R	+	−	−
	L. D.	+	+	−
	L. D.	+	+	+
Gojra	S. P.	+	−	+
Toba Tek Singh ...	S. P.	+	+	−
	C. R.	+	+	−
Sargodha	S P.	+	+	−
Sillanwali	S. P.	+	+	−
Bhalwal	S. P.	+	+	−
Nankana Sahib ...	S. P.	+	+	+
Jaranwala	S. P.	+	+	+
	S. P.	+	−	−
	C. R.	+	+	−
Tandlianwala ...	S. P.	+	+	−
Sukheki	S. P.	+	+	+
Patti	S. P.	[illegible]	+	+
Jhang	S. P.	+	−	−
Okara	S. P.	+	+	−
Phagwara	S. P.	+	−	+
Kasur	S. P.	+	+	+
Patokki	S. P.	+	+	−
Moga	S. P.	+	+	−
Multan	L. D.	+	+	+
Sukkur	L. D.	+	+	−

* L. D. refers to Messrs. Louis, Dreyfus & Co.
S. P. refers to Messrs. Sanday, Patrick & Co.
C. R. refers to Messrs. Clements Robson & Co.
+ = Present. − = Absent.

The most noticeable features about this table are the frequency with which *A. undulatus* occurs (specimens being received from every place), and the comparative scarcity of *C. oryzæ*. It also points to the conclusion that *A. undulatus* and *R. dominica* have a wider distribution in the province than *C. oryzæ*. In considering these results it must however be remembered that they cannot be taken in any way as final, but they are useful in indicating the occurrence of the insects.

THE LIFE-HISTORIES OF THE INSECTS FOUND IN STORED WHEAT.

Before giving the details of the life-histories of the insects dealt with, it will be useful to give some idea of the way in which the observations were carried out. A criticism often applied to entomological investigations is that the results, which have been obtained by observations in the laboratory or insectary, are not always of the value which they should be, owing to the difference which exists between the natural conditions and those under which the observations were made. With this investigation, however, the difference between the natural conditions and the laboratory conditions is not so very marked with the possible exception that the diurnal variation in temperature in a *kotha* or large bin of stored wheat would not be as great as that of the laboratory. This was compensated for by keeping the insects in a closed chamber (an incubator with a water jacket was used but no artificial heat of any kind was employed) so that the temperature would not vary as much as the laboratory itself during the day but the seasonal variation would still be operative. The chamber served another purpose in protecting the insects under observation from accident, and also from the frequent dust storms which are such a nuisance during the hot weather in the Punjab. The insects under observation were isolated in small porcelain crucibles such as are in common use in chemical laboratories, and each was examined daily and notes recorded. In describing the life-histories of the insects, the three which, as has been shown above, are the most important agents in causing damage to the wheat will be taken first and the other insects of lesser importance later.

Attagenus undulatus, Motsch. (Frontispiece).

Although this insect is so common in the Punjab, and of such importance as a pest of stored wheat, that it has attracted the attention even of the native grain dealers in that they have given it a distinctive name in the vernacular, *viz.*, *khapra*, yet it has received little notice from scientific entomologists.

It is first mentioned in Indian entomological literature as a pest by Cotes (1894) in Indian Museum Notes. In these (Vol. III. No. 3, page 119) in which figures of the larva and the beetle are given, it is stated :—" It is known as 'khapra' in the Delhi bazaar, where it is said sometimes to destroy as much as six or seven per cent. of wheat stored in godowns." Lefroy (1906) mentions the insect in Indian Insect Pests though apparently he does not attach any importance to it as a grain pest but says :—" A dermestid (*Æthriostoma undulata*) closely allied to the last is reported from India in wheat. This or the last is the insect found in grain in Gujerat where it is believed to be of use in checking other grain insects notably the Red grain beetle. This belief is so firmly held that the dermestid is introduced in the grain infected with insects as a check on them." The same author (1909) again records the insect making much the same comment " *Æthriostoma undulata* (Motsch) is found in wheat. Its larva is broad, with short hairs, with no anal tube or hooks. The part it plays in wheat is not ascertained but it is likely to be predaceous upon the other insects there or to feed on their dead bodies."

Investigation has shown that this insect certainly does not play the rôle suggested by Lefroy in his later note, but is a very active agent in damaging the wheat.

A. undulatus is also included by Fletcher[1] (1914) in a list of insect pests of stored products, but no account of it is given as its occurrence has not been definitely noted in Southern India.

The egg (Frontispiece, figures 1-3). The eggs are laid loose among the grains of wheat, and as a rule singly. Sometimes they have been observed to be laid in the groove on the grain and in this case two or three may be laid together. The size and shape of the eggs vary to some extent but a typical egg is narrow, cylindrical, rounded at one end and somewhat pointed at the other; the pointed end having a few hairs upon it. In colour it is a translucent white when laid, and remains so for some time. The surface of the egg-case is smooth. After a while, as development proceeds, reddish, or yellowish brown markings appear from which the form of the developing larvæ can be distinguished. The markings take the form of transverse brown bands, and a brown spot at the rounded end, and are due to the long brown hairs with which the segments of the larvæ are furnished, the spot at the end of the egg marking the long tail hairs which lie coiled up inside the shell.

Compared with other insects which attack stored wheat, *A. undulatus* lays a small number of eggs. The largest number observed was 41 and as

[1] *Loc. cit.*

a general rule each female lays between 35 and 40 eggs. The eggs are not laid with any regularity and generally the female lays a large number the day after fertilization (as many as 20 having been recorded) and then a few each day for the remaining days of its life.

Below, two tables are given showing the number of eggs laid each day by two females :—

TABLE III.

Dates of observations	Number of eggs found
May 2-May 3, 1914	14
May 3-May 4, 1914	3
May 4-May 5, 1914	6
May 5-May 6, 1914	7
May 6-May 7, 1914	7
May 7-May 8, 1914	0 (dead)
TOTAL ...	37

TABLE IV.

Dates of observations	Number of eggs found
Sept. 2-Sept. 4, 1914	2
Sept. 4-Sept. 5, 1914	19
Sept. 5-Sept. 6, 1914	16
Sept. 6-Sept. 7, 1914	0
Sept. 7-Sept. 8, 1914	1
Sept. 8-Sept. 9, 1914	0 (dead)
TOTAL ...	38

The hatching of the egg. The larva emerges from the egg by breaking through the shell at the pointed end and crawling through the aperture thus formed. After the body is free from the shell, the larva often remains for some time with the long tail hairs still inside the shell and then finally releases itself and crawls away. The aperture in the egg-case does not close after the larva has emerged, and the shell itself becomes thrown into a number of close longitudinal wrinkles which give the empty egg-cases a beautiful iridescent appearance.

The period which is occupied between oviposition and the hatching of the egg varies, of course, according to the season of the year, but the variation is not extensive. When the insect is most active, that is, during the hot months, the period is five days. The following table will give an idea of the time taken at different times in the year, during which adult females are found. From October to March the insect remains in a hibernating condition in the larval stage, so that during these months no eggs are produced.

TABLE V.

Date of oviposition	Date of hatching	Period
1914	1914	
11th April	17th April	7 Days.
3rd May	8th May	6 ,,
15th ,, ...	19th ,, ...	5 ,,
12th June	16th June	5 ,,
25th ,, ...	30th ,, ...	6 ,,
13th July	18th July	6 ,,
31st ,, ...	5th Aug.	6 ,,
31st Aug.	5th Sept.	6 ,,
10th Sept.	15th ,, ...	6 ,,

The larva. The freshly hatched larva (Frontis. figure 4) is of a uniform yellowish white with the exception of the head which has a brownish or yellowish brown tinge and the hairs on the segments which are yellowish brown. In a short time the colouration of the body becomes evident and consists in addition to the darkening of the colouring of the hairs and head, of transverse bands of a yellowish brown colour on the segments. The underside of the larva is of a uniform cream colour. The segments are furnished with two kinds of hairs, long simple hairs, and short barbed ones. The long hairs are arranged in groups and project from either side of the segments. The barbed ones are found scattered all over the dorsal surface of the body, but particularly congregated into groups on the posterior margins of the terminal segments. In this (the first instar), the long hairs are very long in proportion to the size of the body and the barbed hairs are comparatively few in number and are only arranged in a group or fringe on the ultimate and penultimate segments. As the larva develops the long hairs become relatively shorter in proportion to the body and the number of barbed hairs increases. In the wheat, these barbed hairs are often found felted together in irregularly shaped brown masses, especially when the wheat has been infected with the larvæ for some time.

The young larva moves about actively in search of food and crawls upon the grains in search of a suitable place to attack. After a period of continuous feeding the larva becomes darker in colour and finally leaves the spot where it was feeding and crawls away to hide itself preparatory to moulting. The larvæ kept under observation invariably crept between the underside of the wheat grains and the bottom of the crucible. In

moulting, the skin splits along the back from the head to the region of the penultimate segment, and the larva crawls out leaving the cast skin behind and then immediately recommences to feed.

In the second instar (Frontis. figure 5) very little difference is noticeable beyond the increase in size. The general colour is a little darker and the number of hairs in the fringes on the terminal segments is greater, giving these a darker brown appearance. These fringes are however still found only on the last two segments.

In the third instar (Frontis. figure 6), again there is merely a general darkening in colour and increase in the number of the barbed hairs in the fringes at the end of the body. The long hairs also have now become reddish brown in colour rather than yellowish brown.

In the fourth instar (Frontis. figure 7) a little more change is noticeable. The body and head are of a general brown colour, except the folds of integument between the segments which still retain the yellowish tinge, and when the larva is extended give it a banded appearance. The most noticeable difference however is that a fringe of barbed hairs has now appeared on the third segment from the end.

In the fifth instar (Frontis. figure 8) a further change has taken place. A fringe of barbed hairs has now appeared on the fourth segment from the tail end. Beyond this and the increase in size this stage closely resembles the previous one.

In the succeeding instars these fringes of barbed hairs do not extend any further up the body but the number of hairs in each is increased with each moult, giving the terminal segments a very dark appearance. In the case of a larva, which had moulted ten times (December, 1914), these hairs had assumed the form of thick brushes increasing in density from the fourth terminal segment to the tail. The appearance and colour of the hinder end of the body is therefore an indication of the age of the larva.

The number of times that the insect casts its skin varies according to the season, and also according to the sex of the resulting adult. In fact no definite number can be stated as the typical number of instars for any particular time of the year, for considerable variation may occur even among the progeny of the same parent. It is difficult to see what conditions control the number of moults, for all the insects kept under observation were reared under what appeared to be identical conditions in every respect and yet variation even among the progeny of the same parents would occur. For example, of two

larvæ hatched from eggs laid on the same day (19th May 1914) by the same female, one passed through seven moults, pupating on June 22nd. and the other through four moults pupating on June 8th. It is true that in this case the resulting adults were of different sexes, the former being a female and the latter a male, and as a general rule the female-producing larvæ pass through one more moult than the male-producing ones, yet it is interesting from the biological point of view that two eggs from the same female, reared under the same conditions, should give such different results. As has been said above, the season of the year is also a factor in determining the number of moults. During the hot dry months of May, June and part of July the insects developed very rapidly and the number of moults was reduced, the smallest number observed being four. The general number during the period was six for the females and five for the males. After the advent of the rains the number of moults increased and development was considerably prolonged. Here again considerable variation was observed. When the insect is active the interval between the moults is generally about five days. Later on in the year, after the rains have fallen, the period becomes gradually more extended until the larva passes into a state of hibernation when the period becomes very extended indeed, often to as much as five months. On the next qage are given a few examples illustrating the variation in the number of moults passed through by different insects :—

TABLE VI.

Sex of adult	Date of hatching	Date of first moult	Date of second moult	Date of third moult	Date of fourth moult	Date of fifth moult	Date of sixth moult	Date of seventh moult	Date of eighth moult	Date of ninth moult	Date of tenth moult	Date of eleventh moult	Date of twelfth moult	REMARKS
	1914	1914	1914	1914	1914	1914	1914	1914	1914	1914	1914	1915	1915	
♂	Apl. 17	Apl. 25	May 1	May 6	May 13	May 16	May 23	May 31	June 3					
♂	May 19	May 23	,, 30	June 3	June 8									Eggs obtained from the same female and laid on the same day.
♀	,, 19	,, 23	,, 27	May 31	,, 3	June 9	June 18	June 22						
♂	June 4	June 9	June 13	June 16	,, 20	,, 25								Ditto.
♀	,, 4	,, 9	,, 12	,, 16	,, 20	,, 25	July 1							
♂	July 11	July 15	July 18	July 22	July 27	Aug. 2								
♀	,, 18	,, 22	,, 27	,, 31	Aug. 5	,, 12	Aug. 19	Aug. 27	Sept. 6	Sept. 16	Sept. 29	Apl. 3	Apl. 22	
♂	Aug. 5	Aug. 12	Aug. 16	Aug. 20	,, 24	,, 29								Ditto.
♀	,, 5	,, 11	,, 16	,, 20	,, 24	,, 30.	Sept. 5							
♂	Sept. 6	Sept. 10	Sept. 15	Sept. 21	Sept. 29	Oct. 19	Apl. 3 1915.	Apl. 24 1915.						

The pupa. In common with other Dermestidæ, the insect pupates enclosed in the last larval skin (Frontis. figures 10 and 11). In the last ecdysis the larval skin splits along the back from just behind the head to the region of the penultimate segment but the skin is not cast off and the dorsum of the pupa protrudes through the aperture formed by the slit. The shape of the skin is distorted somewhat, the thorax of the pupa being much wider than that of the larva, so that the aperture in this region is much wider. The dorsal surface of the pupa seen through this aperture is covered with hairs and the outlines of the portions of the body are not very marked. The hairs along the median line are often arranged to form a distinct ridge, and the colour is of a lighter brown than that of the larva. Seen from the underside, however, all the parts of the future beetle are clearly marked. The general colour of the abdomen is a pale yellowish brown, the wing cases, appendages and head being darker in colour. The eyes are distinctly marked. Figures 10 and 11 (Frontispiece) give an idea of the pupa from the dorsal surface, whilst still enclosed in the larval skin and figure 12 is a drawing of the pupa from the ventral surface, and removed from the larval skin. A noticeable feature about the pupæ is their difference in size, the pupa of the male beetle (figure 10) being markedly smaller than that of the female (figure 11).

When the beetle emerges from the pupa case, this is pushed away to the tail end of the enclosing larval skin, but the beetle still remains enclosed in the larval skin until colouration develops and the insect matures. This may take two or three days.

The adult. A. undulatus is a small, active, brownish black beetle, not much longer than it is broad, the head small, compared with the rest of the body and the division between the thorax and abdomen not marked. The colouration in the female is lighter than that of the male. In the male the head and thorax and tip of the abdomen are of a dark brownish black, the elytra and the appendages of a lighter colour. In the female the difference in colour between the head and thorax and the elytra is not so marked. The eyes are black. The whole of the surface of the body and elytra is covered with fine light-coloured hairs, giving the body almost a velvety appearance. A fringe of brown hairs covers the tip of the abdomen. The females are easily distinguished from the males by their larger size, and there is also a difference in the shape of the antennæ in the two sexes. In the male the last joints are more elongated and the basal joints are also different (figures 15 and 16).

The insects in the adult stage have never been observed to damage the wheat. The whole function of this stage is the production of fertile eggs and

when this is accomplished the adults die. The beetles, however, are furnished with well developed mouth-parts so that *a priori* they should feed on something and they have been observed sometimes to gnaw away at the surface of the wheat grains which have already been eroded by the larvæ. Food does not, however, seem to be necessary for the production of eggs, for in several instances fertilized females gave the normal number of eggs without being supplied with any food whatever. The adult beetles live for a short time compared with the larvæ, the longest observed period being ten days.

The females only require to be fertilized once to produce the full number of eggs. During copulation the male remains at the side of the female, the tips of their abdomens approximating. The pair only remain together for a short time, the time in three cases observed varying from one to one-and-a half minutes. The males are capable of fertilizing more than one female.

The length of life-history. The Dermestidæ are characterized by a great adaptability to circumstances and this insect furnishes a good example of the phenomenon. The most advantageous conditions for its development are found in the hot and dry weather which occurs in the Punjab during the months of May and June and part of July. During these months the insect is very active and the life-history is short, the shortest period observed being twenty-five days for males (8th June to 2nd July) and thirty-three for females (9th June to 11th July). With the advent of the rains, however, the life-history lengthens, being about 30 days for males and 40 for females (July and August). From September onwards the conditions seem to be inhibitive to complete development and the insect remains in the larval stage in a condition of partial or complete hibernation. During September and October the larvæ moult fairly regularly but with lengthened periods between the moults, but from October onwards they remain completely dormant. This goes on until about the beginning of April of the succeeding year when the larvæ again exhibit activity. In the case of the isolated insects kept under observation during this period they remained between the under surface of the wheat grains and the bottom of the containing crucible. It was noticed, however, in the case of a large number of larvæ which were kept in a vessel with a quantity of wheat, that they forced their way down among the grains clustering together as if for warmth, whereas, when active, they are mostly to be found on the surface of the wheat. Under natural conditions the larvæ have been found in various places during the hibernating period. Where the wheat has been stored in godowns or *kothas* (native storehouses) the larvæ are almost invariably to be found in the cracks and crevices between the bricks or in the plaster on

the walls. In one instance over a hundred larvæ were extracted from a crack, roughly an inch and-a-half long and an inch deep, in the plaster on the wall of a *kotha* and as the walls were full of such cracks and each one seemed to contain larvæ, this will give some idea of the number of larvæ which were hibernating in the place. Where the wheat is stored in gunny bags the larvæ often remain in the bags among the wheat.

It will be seen, therefore, that this insect when it meets adverse conditions merely passes into the dormant state, and continues so until conditions change. It follows, from this habit of hibernating in the larval stage, that from October to April no increase in the numbers of the insects occurs, as during this period no adults are to be found. Below is given a number of examples of the period of life-history of the insect at different times during the year. For the sake of convenience the table commences with the month of April as it is at the beginning of this month that activity commences :—

TABLE VII.

Date of oviposition	Date of emergence of adult	Sex	Period
1914	1914		
11th April ...	6th June	♂	57 Days.
15th May ..	25th ,,	♀	42 ,,
15th ,, ...	12th ,, ..	♂	29 ,,
8th June ...	2nd July ..	♂	25 ,,
9th ,, ...	11th ,,	♀	33 ,,
13th July ...	24th Aug. ...	♀	43 ,,
16th ,, ...	17th ,, ..	♂	33 ,,
31st Aug. ...	29th April 1915 ...	♀	242 ,,
10th Sept.	30th ,, ,, ..	♂	233 ,,

The number of generations. For this purpose it will be as well to consider the year as beginning with April and ending with the following March, for eggs are not usually available until the middle or end of April. This being so, it may be said that there are four generations in the year, the first three, which occur during the period of greatest activity, being the shortest. The fourth has a very extended period as it occurs during the period of hibernation, and extends from September until the following April or May. In the example given, the first generation is considered as commencing in the beginning of May.

In obtaining these results the periods given are necessarily those of the females but in many cases the males matured in a much shorter time.

TABLE VIII.

Number of generation	Date of oviposition	Date of emergence	Period
	1914	1914	
First	13th May	19th June	38 Days.
Second	22nd June	26th July	35 ,,
Third	31st July	7th Sept.	39 ,,
Fourth	10th Sept.	30th April 1915	233 ,,

The effect on the grain. The larvæ of *A. undulatus* attack the grain in a characteristic way. Passing, as they do, the greater part of their life outside the grains, the damage that they cause takes the form of a gnawing away or erosion of various parts of the grain. No particular part of the grain seems to be especially attacked, but it has been determined by experiment that the attack always begins at some weak place in the pericarp. It was observed occasionally that larvæ bored their way into the grains at the place where the primary attack was made, but on the other hand other larvæ merely gnawed away at the exposed surfaces of the grain, and a wheat dealer at Ludhiana informed the writer that he had noticed that wheat attacked by this insect was often white due to the fact that the larvæ had gnawed away the yellow pericarp.

During the investigation it was considered necessary to determine whether the larvæ in their varying stages could attack whole grains of wheat. Accordingly a quantity of larvæ was reared on grains which were already damaged. Then a number of larvæ in each of the first to the fifth instars was isolated with whole grains of wheat which were carefully examined to see that the pericarp was intact. It was found that in the first, second and third instars the larvæ were unable to penetrate into entire grains but in the fourth instar, that is after the third moult, they could do so. In practice, however, this fact would not have very much significance except perhaps in the primary attack, but even then there would be enough damaged grains for the freshly-hatched larvæ to penetrate. Moreover, the primary attack is in all probability made by larvæ which have passed the third instar and have been hibernating through the cold weather and the grains which they might damage would provide sufficient food for the early stages of the succeeding generation.

Another interesting feature of its method of attack is the prevalence of the insect at the top of the wheat. It certainly can and does penetrate to

some depth into the wheat, but the greatest amount of damage always occurs in the first six to twelve inches, and during the first year. The damage caused by the insect very soon becomes obvious owing to the collection of cast larval skins seen lying about the surface of the grain, and when the attack becomes bad, the quantity of these skins and other detritus which collects on the surface of the wheat is extraordinary.

The larvæ of *A. undulatus* unlike the adults of *R. dominica* and *C. oryzæ* consume all the material they gnaw from the grains, and wheat attacked by this insect does not become mixed with the floury matter which characterizes the attack of the other two insects. A quantity of dust and frass is produced but this consists almost entirely of the excrement, which is composed of small white or yellowish white pellets. This is quite characteristic and often indicates the cause of the damage to the wheat when no insects are to be found.

Although the normal food of the larvæ is the wheat grains, yet they can live on flour or the floury matter which is often found amongst wheat, and several larvæ have been reared on this material. This may possibly be resorted to by the young larvæ when they are unable to find grains which are suitable for them to attack.

Another curious fact regarding this insect is that wheat which is badly attacked by it, always feels hot to the touch, and it was found that the temperature of a number of larvæ collected together was considerably above the air temperature.

Rhizopertha dominica, Fb.

Like *A. undulatus* this insect has not received much attention in India but in America its importance as a pest of stored grain has been recognized, and an account of it was given by Chittenden[1] (1911). It has also been recorded by other observers in other countries. This author (Chittenden) gives a short bibliography. In the literature on Indian economic entomology it has not received much attention. Records of the occurrence of the insect were given at different times in Indian Museum Notes by Cotes[2] (1889-1894). Stebbing[3] (1903) also gives a short description of the insect and a few notes

[1] Chittenden, F. A. The Lesser Grain Borer (*R. dominica*). *Bull., U. S. Dept. Agri. Bureau of Entomology*, 96, Part III, pp. 29—47, figs. 7 and 8.

[2] Cotes, E. C. Notes on *R. dominica*. *Indian Museum Notes*, Vol. I, 1889, p. 60.
" " " " , Vol. II, 1889, p. 27.
" " " " " Vol. II, 1893, p. 150.
" " " " " Vol. III, 1894, p. 124.

[3] Stebbing. Note on *R. dominica*, *Indian Museum Notes*, Vol. VI, pp. 25 and 26.

on its life-history. Lefroy (1906) does not mention it in Indian Insect Pests and merely mentions it as a household pest in Indian Insect Life (1909). Fletcher[1] (1914) records it as a minor pest and gives some details of its life-history. None of these authors, however, gives any complete data of the bionomics of the insect so that information on these points, which are of importance in studying the insect as a pest, had to be worked out afresh.

The egg. (Plate II, figures 1 and 2.) The egg has been described by Chittenden as :—" White, of elongate pear-shaped form, one end forming a rather narrow stem or neck, bearing on one side at its base a transverse impression or suture, causing the egg to bend somewhat to one side. Both ends of the eggs are rounded : the surface is slightly polished and apparently somewhat rough. The length of the egg is 0·6 mm. and across its thickest portion a little over 0·2 mm."

A little variation from this description has been noticed, the eggs when they are freshly laid are perfectly white, but after a short while a pinkish tinge appears particularly towards one end, and this is the characteristic colour of the eggs. The shape too is rather more cylindrical than pear-shaped, and the narrow neck or stem is really a small peduncle formed by a pulling out of the end of the egg-case during the process of oviposition.

Each egg is laid separately and either loose among the grains or in some cases stuck to them. It has also been noticed that the eggs are sometimes laid in clusters and not singly. This nearly always takes place when a large number of eggs is laid on the same day, as often occurs. The process of oviposition was observed in one instance. The ovipositor of a female which had eaten its way right inside a grain of wheat was thrust out through a hole in the grain. The ovipositor became very much elongated, when fully extended being nearly as long as the abdomen, and then became tense and swollen due to the passage of the egg into its lumen. Sometimes the internal pressure was so great that the ovipositor became coiled upon itself. The egg then passed into the posterior portion of the organ and was then rapidly extruded, but the egg was held for some time in the distended orifice and the organ waved about as if seeking for a place in which to deposit the egg. After a short interval the egg was released. The eggs were laid in rapid succession, following one another quickly down the organ.

For its size this insect lays an extraordinary number of eggs and the period during which it is actually ovipositing may be an extended one. The largest number observed is 586 and the general number is between 300 and 400.

[1] *Loc. cit.*

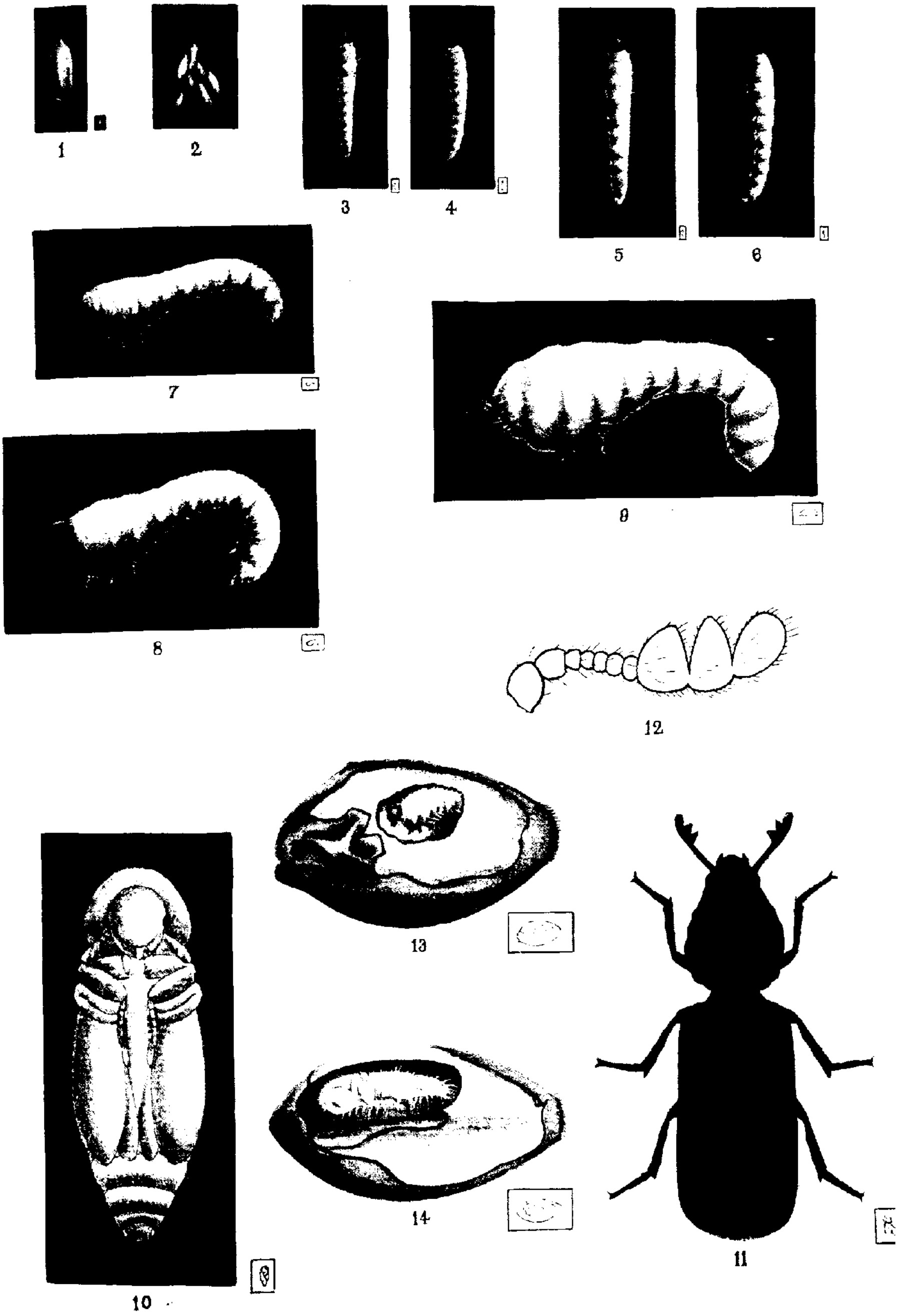
PLATE II.

Rhizopertha dominica.

Fig. 1. The egg (× 20).
,, 2. A cluster of eggs (× 10).
,, 3. A freshly emerged larva, dorsal view (× 26).
,, 4. A freshly emerged larva, lateral view (× 26).
,, 5. The larva after the first moult, dorsal view (× 26).
,, 6. The larva after the first moult, lateral view (× 26).
,, 7. The larva after the second moult (× 26).
,, 8. The larva after the third moult (× 26).
,, 9. The fully developed larva just previous to pupating (× 20).
,, 10. The pupa. Ventral view (× 20).
,, 11. The adult showing the attitude when actively moving about (× 20).
,, 12. The antenna.
,, 13. A grain showing the larva inside (× 5).
,, 14. A grain showing the pupa in the cavity excavated by the larva (× 6).

The small figures by the side of the larger ones indicate the natural size of the insect

PLATE



RHIZOPERTHA DOMINICA.

Below is given the number of eggs laid by several females and the periods during which each insect laid the eggs :—

TABLE IX.

Date of commencement of oviposition	Date of death	Number of eggs laid	REMARKS
1914			
28th May ..	....	383	Ceased laying on 27th July 1914.
6th June ..	8th August 1914 ...	320	Ceased laying on 22nd July 1914.
9th July ..	23rd April 1915 ...	586	Ceased laying eggs on 18th November 1914.
10th ,, ...	7th August 1914 ...	209	Ceased laying on 3rd August 1914.
11th August ..	....	480	Still alive on 4th May 1915. 3 eggs laid on 30th April.
22nd ,, ..	....	381	Still alive on 4th May 1915. Ceased laying on 22nd September.

The time taken to produce this number of eggs is considerable. For instance, one female commenced to lay eggs on 9th July 1914 and continued to do so until 21st August when it ceased laying but still continued to live. Up to this time it had laid 349 eggs. On 4th September it was still alive and another male was placed in the crucible with it and allowed to remain until the 18th when it was removed and it was found that the female had laid 9 eggs. From this date it continued to lay eggs until 18th November when it ceased to lay but continued to live on, having burrowed its way into a grain of wheat where it remained quiescent. It continued to live in this condition until 23rd April 1915 when it died without having laid any more eggs. In all it had produced 586 eggs.

It would appear from this that one fertilization does not suffice to supply enough spermatozoa to fertilize all the eggs which a female is capable of laying. It has also been found that one male would copulate at least twice. The number of eggs laid per day varies very considerably. It has been noticed that previous to the commencement of oviposition the female feeds vigorously, producing a quantity of the floury frass which is characteristic of the attack of this insect, and then as a rule a large number of eggs is laid daily for some time. Then comes a period of less activity coincident with increased feeding, and this is followed in its turn by a larger daily output of eggs. This is

exemplified in the following table which is the daily oviposition record of a female for one month :—

TABLE X.

Date of observation. 1914	Number of eggs laid
June 1—2	20
,, 2—3	...
,, 3—4	12
,, 4—5	10
,, 5—6	...
,, 6—7	8
,, 7—8	4
,, 8—9	...
,, 9—10	6
,, 10—11	19
,, 11—12	23
,, 12—13	19
,, 13—14	21
,, 14—15	3
,, 15—16	...
,, 16—17	22
,, 17—18	7
,, 18—19	15
,, 19—20	10
,, 20—21	...
,, 21—22	10
,, 22—23	12
,, 23—24	6
,, 24—25	...
,, 25—26	...
,, 26—27	13
,, 27—28	14
,, 28—29	16
,, 29—30	14
TOTAL ...	284

Oviposition continued steadily until the end of September in some cases but in others it continued until the middle of November, after which all oviposition ceased. The females, however, did not die but lived on, usually buried in the inside of the grain of wheat which they had attacked, and evidently in a hibernating condition for their movements were sluggish and they did not seem to feed much.

The hatching of the eggs. Very little change takes place in the appearance of the eggs until they are nearly ready to hatch when they assume a more opaque white appearance and lose the reddish tinge. The larva emerges by breaking through the egg shell at the end and often remains on the empty shell for some time. The empty egg shell has a collapsed appearance and is of an opaque white colour. As a rule the larva leaves the egg shell untouched

after emerging, but instances have been noticed where the empty egg shells have been partially devoured by the young larvæ. The period, which elapses from the laying of the eggs to the appearance of the larva, varies of course according to the season but during the most active period (May-August) it is usually five days. This is a much shorter period than that given by Chittenden[1] (1911) (14 days, April and May) and Fletcher[2] (1911) (10 days, season not stated). In the United States the period is likely to be more prolonged because of the more temperate climate, but it is difficult to understand the extended period given by Fletcher, as the climate of Madras should be such as to provide optimum conditions for the development of the eggs.

With the advent of the cold weather the period required for the eggs to hatch increases very rapidly. From a reference to the table given below which shows the period required for the hatching of the eggs from May to November, it will be seen that although the period remained fairly constant till the end of September, yet during October and November the period increased from 11 to 26 days. These latter periods were obtained from eggs laid by a female which had continued to lay until this time although the majority of the females under observation had ceased to oviposit in September.

TABLE XI.

Date of oviposition	Date of hatching	Period
1914.	1914.	
17th May ...	21st May	5 Days.
29th ,, ...	2nd June	5 ,,
10th June	15th ,, ...	6 ,,
7th July	12th July	6 ,,
19th ,, ...	24th ,, ...	6 ,,
12th August	17th August	6 ,,
22nd ,, ...	27th ,, ...	6 ,,
14th September	19th September ...	6 ,,
30th ,, ...	6th October	7 ,,
17th October	27th ,, ...	11 ,,
5th November ...	22nd November ...	18 ,,
15th ,, ...	10th December ...	26 ,,

The larva. Chittenden[1] (1911) gives the following description of the newly hatched larva :—

" The newly hatched larvæ were described as white, slightly yellowish towards the head, head yellowish, ocelli reddish brown, arranged in a triangle, mouth parts brownish, antennæ very short, the head beset with a few long hairs, legs tolerably long, slightly yellowish with long claws. Each of the

[1] *Loc. cit.* [2] *Loc. cit.*

abdominal segments bears ventrally a number of long hairs and similar hairs are also on the dorsal side of segments 7 and 8. The last segment bears a slightly curved yellow horn, directed backwards. Length a little less than 0·3 mm."

Little needs to be added to this description. The freshly hatched larva (Plate II, figures 3 and 4) is very active and moves quite rapidly about the grains. In this stage, it does not assume the characteristic curved form of the Bostrychid larva, nor is the thorax markedly enlarged.

In the second instar (Plate II, figures 5 and 6) it still retains the shape of the freshly emerged larva and practically the only noticeable difference is the increase in size.

In the third instar (Plate II, figure 7) the larva has now commenced to assume the typical curved form, and the thorax has become much more swollen. There is still no noticeable change in colour.

In the fourth instar (Plate II, figures 8 and 9) the larva reaches its full development. Chittenden's[1] (1911) description of the larva in this stage is as follows :—

" The larva when fully developed is of the characteristic Bostrychine form similar to that of *Dinoderus truncatus*. It is rather more elongate than usual in the *Ptinidæ* and more constricted in the middle. The ground colour is white, the head light brown, and the mandibles dark brown, nearly black. The claws of the legs are light brown. The body is covered with minute slender pale brown hairs, which are denser and somewhat longer on the first thoracic and last two abdominal segments.

" The larvæ when lying on their sides somewhat resemble on a smaller scale those of the *Lamellicorns*, the body being curved in the same manner ; locomotion in this position is possible but very slow.

" Full-grown larvæ measured about 2·8 mm. in length."

The larva passes through four moults as a rule though one or two cases have been observed where five moultings took place. These results were obtained by rearing the larvæ on flour as of course daily examination of larvæ inside grains would be difficult and the frequent disturbance due to the cutting open of the grain would not have a good effect on the larvæ. Larvæ living exposed in this way took a little longer to pass through their metamorphosis than those which lived inside the grains, this being probably due to the artificial nature of their surroundings. The experiment was also useful in showing that the larvæ can live and mature in floury material of this kind, a point which will be discussed later. The intervals between each successive moult were fairly regular after the first one, but this was usually

[1] *Loc. cit.*

rather extended. This may, however, have been due to the somewhat artificial conditions under which the larva was living rendering it perhaps more difficult to get through the initial stages, but when it had become well established and the first moult safely passed through, then development would proceed faster.

The pupa. The pupa is at first quite white but later brown pigment is laid down in the eyes and trophi. The pupa exhibits the characteristic depressed head and enlarged thorax of the adult. The dorsal surface is covered with hairs.

The pupa lies in the cavity inside the grain which the larva has excavated during its feeding processes. No cocoon of any sort is prepared, and the exuvia and excrement of the larva are pushed into the unoccupied space left in the cavity of the grain at the tail end of the pupa. In the case of the larvæ which were reared upon flour, an elliptical cavity was hollowed out by the larva in the floury material in which it pupated.

The adult. Chittenden[1] (1911) describing the beetle says :—" The beetle is about one-eighth inch long and about one-thirty second of an inch in width, quite narrow being therefore approximately four times as long as wide. The form is nearly cylindrical, the head is comparatively large and prominent and bent down under the thorax in the manner peculiar to most members of the family Bostrychidæ ; the antennæ are also prominent, as are the eyes and the mandibles. The antennæ are ten jointed and terminate in a three jointed club. The colour is dark brown or castaneous and polished throughout."

To this description might be added that the anterior margin of the dome shaped thorax is crenulate and that the surface of the thorax and the elytra is pitted ; the pits on the elytra being arranged in lines giving them a striated appearance.

Viewed from above, the head of the beetle is completely hidden, when at rest, by the over-hanging anterior margin of the thorax, but when the insect is active, the head is raised and protruded to some extent, so that the mandibles and a portion of the head appear in view as is represented in figure 11, Plate II.

The males and females are to all outward appearances exactly similar. There is no noticeable difference in size or in the shape of the antennæ. It is therefore extremely difficult to determine the sex of the newly emerged insects. In carrying out the work on the number of generations passed through, it became imperative to know which were females, and this was done in the following way :—A number of insects was selected from the stock and isolated in tubes. As the insects are very active in the natural state, the

[1] *Loc. cit.*

probabilities are that a female very rarely escapes fertilization and by selecting fairly young insects, which can be determined from their colour, those beetles which laid eggs were females and those which did not were assumed to be males. The newly emerged beetles were then tested with these males and in each case by taking a sufficient number it was possible to obtain fertilized females to carry on the generations.

The adults both male and female are provided with wings which they use, and beetles have often been caught on the wing in storehouses and other places where the insects were present in numbers. This is the only one of the three important Punjab stored wheat insects which the writer has caught flying, although the other two possess wings. The extent to which it uses its power of flight is difficult to determine. It has usually been seen flying in an aimless kind of way about rooms and storehouses where wheat is stored, but has not been taken in the field, though there seems no reason why it should not infect piles of wheat which often lie about in villages near to the store places for some time before the wheat is actually stored. In any case it is probably a fruitful source of infection from one godown or *kotha* to another. This is especially so when they are built close together as is often the case in villages and *mandis*, and particularly so, when one considers the indifferent state of repair in which these buildings are often kept, so that intercommunication between the several rooms or chambers is very easy and would afford no obstacles to the passage of such a small insect as *R. dominica*.

The adult after emerging from the pupal covering remains generally for a few days inside the grain in which it has developed, until it is mature, and then cuts its way out through the side of the grain.

The length of life-history. This of course varies with the season of the year, but the insect is most active from May until August. From September onwards the period of life-history lengthens and in December, January and February the insect merely "marks time," remaining in a hibernating condition in whatever stage it was when the cold weather set in. The shortest period observed was 24 days (June and July 1914) and the average period during the time of general activity is 27 days. This period is again shorter than given by Chittenden[1] (1911) (108 days April 27—August 12) and Fletcher[2] (1914) (probably less than two months) but neither of these observers had determined the period exactly. It is, however, difficult to understand why Fletcher should have suggested such an extended period, for there does not seem to be any reason why the average period in Madras should not be nearly the same as in the Punjab.

[1] *Loc. cit.*
[2] *Loc. cit.*

The following table gives the length of life-history for each month in the year during which the insects are active. For the sake of convenience the table commences with the month of May as this is the beginning of the period of activity. The figures given are the actual number of days which elapsed from the laying of the egg to the emergence of the beetle from the pupa case. This last was determined by cutting open the grains about the time when pupation should have taken place, for, as has been mentioned previously, the adult in nature usually remains inside the grain for some days after emerging from the pupa case before cutting its way out :

TABLE XII.

Date of oviposition	Date of emergence of adult	Period
1914	1914	
27th May	20th June	35 Days.
29th ,,	1st July	32 ,,
3rd June	3rd ,,	31 ,,
25th ,,	18th ,,	24 ,,
10th July	4th August	26 ,,
12th August	8th September	28 ,,
22nd ,,	19th ,,	29 ,,
14th September	20th October	37 ,,
23rd ,,	13th November	52 ,,
6th October	6th April 1915	183 ,,

The number of generations per annum is probably five. In carrying out the investigation to determine the number of generations, some days were allowed to elapse between the successive generations to compensate for the period during which the insect usually remained inside the wheat grain in which it had developed, before breaking its way out. Below is given a typical series of generations :—

TABLE XIII.

Generation	Date of oviposition	Date of emergence of adult	Period
	1914	1914	
First	1st April*	17th May	41 Days.
Second	4th June	7th July	34 ,,
Third	11th July	7th August	28 ,,
Fourth	22nd August	18th September	28 ,,
Fifth	6th October	6th April 1915	183 ,,

* This date is only approximate. The larva emerged from the egg on 7th April.

The effect on the grain. The attack of *R. dominica* is quite characteristic, and with a little practice can be distinguished from that of *A. undulatus* or *C. oryzæ*. The most noticeable part of the damage is that effected by the adults. Grains are seen in which the whole of the starchy interior has been eaten away leaving the pericarp of the seed empty and riddled with large

irregularly shaped holes. A large amount of white floury frass soon accumulates, for the insect spoils almost as much, if not more, of the grain than it consumes by reducing it to the condition of flour which, as has been mentioned previously, probably affords a means of support for the young larvæ until they can find grains suitable for them to bore into.

As with *A. undulatus*, experiments were made to determine whether the freshly emerged larvæ could attack and bore into perfect grains, or whether they could only attack those which were in some way damaged. The results of these experiments, although one or two exceptions occurred, go to show that the newly hatched larvæ cannot enter a wheat grain which has the pericarp complete, but the smallest crack or abrasion in the pericarp which has removed the hard outer skin so that the softer underlying tissues can be reached, seems to be sufficient to enable the larva to make its way into the grain. If, however, the pericarp is entire, even fairly mature larvæ are unable to penetrate into the grains. For example three larvæ, one month old which had been reared on frass, were placed along with three grains of which the pericarp was entire, and they failed to enter the grains and ultimately died; two, three days after being put in, and the remaining one on the fourth day. In another instance out of four larvæ which had been feeding on frass for seven weeks, only one succeeded in penetrating a grain and the others died without having done so.

From repeated observations it was found that the freshly hatched larvæ had no difficulty in entering grains which had been sliced, or the pericarp removed in any way, and in rearing larvæ in the investigations upon the life-history of the insect, a small hole was made in the grain with a needle to afford an easy ingress for the larva. Under natural conditions although the young larvæ may find some difficulty in discovering grains which they can penetrate, yet the activities of the adults must render a large number of grains liable to attack by the larvæ, and in this the males would be as effective as the females; and more than this, the frass, which the attack of the adults produces, would afford a means whereby the young larva would be able to tide over the earlier stages, if it was not successful in finding a suitable grain to attack at once.

Calandra oryzæ, Linn.

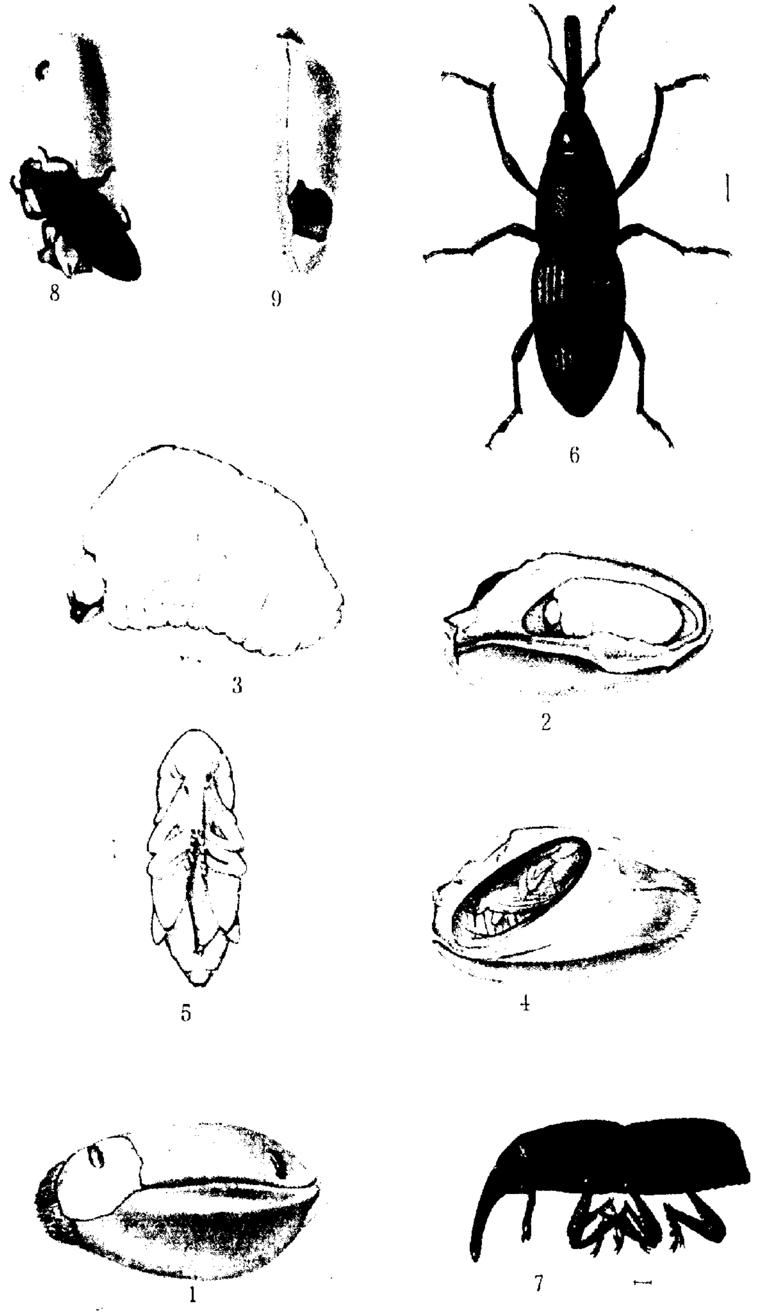
PLATE III.

Of all the insects which attack stored wheat *C. oryzæ* has perhaps received most attention from entomologists owing to its practically world-wide distribution and its importance as a granary pest in other countries. It is not proposed

PLATE III.

Calandra oryzæ

Fig. 1. **Eggs laid on a wheat grain** (× 8).
,, 2. **Larva feeding inside a grain** (× 8).
,, 3. **Larva removed from grain** (× 16).
,, 4. **Pupa in natural position inside grain** (× 8).
,, 5. **Pupa removed from grain, ventral view** (× 16).
,, 6. *C. oryzæ* **from above** (× 16).
,, 7. *C. oryzæ* **from side** (× 16).
,, 8. **Beetle gnawing into wheat grain** (× 8).
,, 9. **Adult inside a wheat grain** (× 8).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here to summarize what has been previously written about this insect by observers in other countries, as much of this literature is not available here; but it will be useful to refer to what has been done with this insect by Indian observers as recorded in the rather scanty literature on Indian economic entomology. The earliest paper of value is that by Cotes[1] (1888) which gives a short account of the life-history of the insect and discusses various remedies. Lefroy[2] (1906 and 1909) gives some details of the life-history of the insect. Fletcher[3] (1911 and 1914) gives more details of its life-history and discusses the relation of dryness and moistness to the development of the insect. Although therefore quite a lot is known about this insect in other parts of India, very little is known with regard to its habits in the Punjab, so that for the purpose of this investigation only those points which are of importance to the problem from the point of view of the conditions which obtain in the Punjab have been considered.

The egg. The egg as described by Fletcher[3] (1911) is laid in a groove or pit prepared for it by the female in the surface of the grain. Any situation on the grain may be selected by the female and it has not been observed, as stated by Fletcher, that special preference for the hairy apex of the grain is shown. Under ordinary circumstances the eggs are always laid buried in the grain but eggs have occasionally been found laid exposed on the wheat grain. These did not hatch, however.

The number of eggs laid by this insect under Indian conditions has never been determined. Hinds and Turner[4] (1911) in their account of the insect give the maximum number observed as 417 and the maximum number laid in twenty-four hours as 20. These authors do not say by what method the number of eggs was determined, whether the eggs were cut out daily or not. It was found too laborious to cut out the eggs in each case, nor was counting the scars made upon the surface of the grains reliable, as every scar did not always denote the presence of an egg. The following method was therefore adopted, which it was hoped would give a fair approximation to the number laid. A virgin female was taken and confined with an active male and supplied daily with 20 fresh grains of wheat; the old ones, in which the female had presumably laid eggs, were then removed, placed in a test tube, and kept under favourable conditions for a sufficient time to allow the eggs to develop into

[1] Cotes, E. C. A preliminary account of the wheat and rice weevil in India. *Notes on Economic Entomology.* No. 1. Government Press, Calcutta.

[2] *Loc. cit.*

[3] Fletcher, T. Bainbrigge. "Weevil and dry wheat." *Agricultural Journal of India.* Vol. VI, pp. 333—343.

[4] Hinds and Turner. *Calandra oryzæ* in Alabama. The life-history of the rice weevil. *Journal of Economic Entomology*, Vol. IV, pp. 230—236, Part 7.

easily distinguishable larvæ. The grains were then cut open and the number of larvæ counted, this number being taken as the number of eggs laid. The results obtained, however, were not very encouraging for the greatest number recorded was 60 between 9th and 31st October 1914, after which date no more eggs were found although the female continued to live for some time afterwards. The largest number recorded in one day was 19. These results are much smaller than those given by Hinds and Turner[1] (1911) and evidently require further investigation.

The larva. The larva has been figured and described several times before by various authors. It is a small legless grub, yellowish white in colour with a yellowish brown head. In its natural state it is slightly curved giving it a humped appearance. Hinds and Turner[1] (1911) state that there are three stages in the larval history, determined by differences in the measurements of the head. This point could not be verified in the same way that the number of moults of *R. dominica* were ascertained, as the larvæ would not live on flour.

The larvæ possess a distinct advantage over those of *A. undulatus* and *R. dominica* for, since the egg is laid beneath the surface of the grain, the larva when it hatches out has a place already provided for it where it may attack the grain, and it has not to search for suitable food as is the case with the other two insects. If it had to do so, as would happen in the cases where the eggs were laid on the surface of the grains, it would be at a distinct disadvantage for, being legless, its scope for locomotion would be very limited.

The pupa. This is a typical curculionid pupa and all the characteristics of the adult can be clearly discerned. Hinds and Turner[1] give the duration of this stage as between three and nine days, and the average as six. Fletcher gives the period as six days in warm weather. In Lyallpur during the time when the insect is most active, that is August and September, the time required for the emergence of the pupa is about 5 or 6 days but as the weather gradually gets colder, this period lengthens and at the end of November may be as long as 14 days. A few examples are given below :—

TABLE XIV.

Date of pupation	Date of emergence of adult	Period in days
1914.	1914.	
9th October ...	14th October ...	6
24th ,, ...	30th ,, ...	7
4th November ...	11th November ...	8
20th ,, ...	3rd December ...	14

[1] *Loc. cit.*

The adult. This is the most easily recognizable of all the insects which infect stored wheat in the Punjab, and is, moreover (with the exception of *C. granaria* which is unimportant, owing to its comparative rarity), the only true " weevil " among them all. Native dealers in some places have learned to distinguish it from the other beetles and call it the *sund wali susri* from its pronounced snout or trunk. It is brownish-black in colour, becoming progressively darker as it becomes older. In its younger stages it may even be quite light brown and is not easily distinguished by its colour from *C. granaria*, but the four reddish brown spots on the elytra and the characteristic pitted appearance of the thorax and elytra render it easily distinguishable.

The sexes are also easily distinguishable, as stated by Hinds and Turner[1] (1911), by the length and breadth of the snout, that of the male being shorter and broader than that of the female.

A curious characteristic of this insect has been noticed. The beetles as a rule lie hidden among the grains, but if the wheat is disturbed, a large number immediately comes out on to the surface of the wheat walking about actively as if seeking to escape from some danger. It has also been noticed that the adults are capable of walking up smooth vertical surfaces with ease, differing in this respect from *A. undulatus* and *R. dominica*.

During copulation the male mounts the female from behind retaining its hold by the front legs the tibiæ of which are armed with strong spines. The females will take the male more than once, as the following example shows: A male and female were put together with some grains of wheat on 21st February 1914. On 1st March they were observed to be coupling, but no eggs were found subsequently. On 7th March they were again seen to be pairing and again on the 13th. The female died on 16th and the male on the 18th and on 28th the grains were cut open but no larvæ or eggs were found. The insects had been kept the whole time in an incubator at 30° Cent. (86° Fahr.) which is an average warm weather temperature, so that the non-production of progeny is difficult to understand.

The length of life-history. No accurate figures are available of the length of time occupied by this insect in passing through its various stages in India. Fletcher[2] (1911) gives some figures, but these cannot be taken as the determination of the actual length of life-history, since they are based upon the dates when the parents were put into the jar of wheat. This, however, cannot be taken as a criterion for the date of oviposition, for the eggs may not have been laid for some days after the parents were put in among the wheat, and there

[1] *Loc. cit.*

[2] *Loc. cit.*

is no reason to suppose that the period between the infection of the wheat and oviposition of the female would be the same in each case. It is clear therefore that these figures cannot be relied upon. A report by Hooper[1] (1913) is quoted by Noël Paton[2] (1913) giving some figures of the length of life-history, but in these again no measures were taken to ascertain accurately the date of oviposition. However, 29 days (May and June) is given as one of the periods observed, and this is considerably shorter than the shortest period given by Fletcher (34-36 days June—July) although the climate of Calcutta is not so very different from that of Pusa.

In Lyallpur *C. oryzæ* behaves in a peculiar manner. During the hot dry weather the majority of the insects die off under ordinary circumstances. At the end of the cold weather a large number of beetles was observed to be leaving a bag of wheat which was badly attacked by them, and to be crawling up the walls of the room in which the wheat was stored. This migration continued until there was scarcely a specimen to be found among the wheat. A large number of these died, but doubtless many of them found suitable lurking places in which to hide until the advent of more favourable conditions. In bottles of wheat in which the insects were allowed to breed and which were being kept to supply insects for other experiments, the insects merely died off, escape from the bottle being impossible. Breeding had apparently ceased entirely, and this was borne out by experiments which were being tried, for at this time of the year all the attempts made to induce the insect to breed were unsuccessful. Even altering the moisture conditions did not have much effect, for a number of pairs of insects were kept in an atmosphere of high humidity, but although this prolonged the life of the insects, breeding only took place in one or two instances, and then not to any large extent. Reducing the temperature and increasing the humidity was more successful. Under natural conditions, however, breeding did not take place until after the rains had come and it was not until the beginning of September that records could be obtained.

This is not the state of affairs which obtains in all parts of the Punjab, for in the moister places (Gurdaspur, Amritsar, etc.), it was found in the beginning of August that *C. oryzæ* had been active for some time, but in these parts the rainfall is higher than that of Lyallpur, and July is usually a wet month; and this would produce conditions favourable to the development of the insect.

[1] Hooper, D. Damage by wheat weevil. Appendix N, *Indian wheat and grain elevators*, by F. Noël Paton.

[2] Noël Paton, F. *Indian wheat and grain elevators.* Government Press, Calcutta,

The way in which this insect reacts towards the conditions of moisture in the wheat has been recorded by Lefroy[1] (1909) and Fletcher (1911),[2] and will be dealt with again subsequently (see page 261 et seq.).

When conditions in Lyallpur were such that the insect could breed under natural conditions, this went on at quite a rapid rate, the period between the laying of the eggs and the emergence of the adult from the pupa case being as short as 22 days. This rapid development went on uninterruptedly until the end of September. In October and November the period required for development lengthened until it had reached a maximum of 50 days. By December and January the period had extended to a maximum of 85 days (2nd November 1914—25th January 1915) and after this, development ceased, the insects remaining in the larval stage in the grains of wheat. The following table gives some examples of the period required for development at different periods :—

TABLE XV.

Date of oviposition	Date of emergence of adult	Period in days
1914	1914	
12th September ...	2nd October ...	21
19th ,, ...	11th ,, ...	23
2nd October ...	29th ,, ...	28
20th ,, ...	4th December ...	46
2nd November ...	25th January 1915 ...	85
3rd December ...	5th April 1915 ...	124

The number of generations. The number of generations in the year is determined by the period during which the conditions are suitable for the breeding of the insect. This varies with different parts of the Punjab. At Lyallpur where the insect begins to breed so late in the year there are probably only three or possibly four generations.

The effect on the grain. As in the case of *R. dominica* the most noticeable effect is that produced by the adults. The effect of the activities of the larvæ on the grains is not easily distinguishable since they do all their work inside the grains. The adults gnaw small almost circular holes into the grains, their long snouts being particularly adapted for the purpose. They do not, however, consume the entire starchy contents of the grains in the way that *R. dominica* does, and in this way the attacks of the two insects can be distinguished.

[1] *Loc. cit.*

[2] *Loc. cit.*

The amount of damage which this insect is capable of doing has been discussed by several writers. Hooper[1] (1913) says that the damage effected by 50 weevils (sexes not stated) and their progeny in 100 grams of hard wheat was a loss of 26% by weight, 65% of the grains being found to be weeviled; the experiment having extended from 12th April to 12th July, 92 days. Fletcher[2] (1911) also states that "a large number of weevils" put into 1 seer (2 lb.) of wheat effected in 167 days a loss by weight of roughly one-third, but makes the reservation that it is difficult to estimate the actual damage. It is clear that the figures obtained from these experiments although of scientific interest cannot be taken as an indication of the amount of damage caused by the insect under normal conditions, since the conditions under which they were carried out do not in any way approximate to those under which the insect usually lives, and the relation between the number of weevils and the quantity of wheat infected is disproportionately exaggerated. In fact it is almost impossible to elaborate an experiment, or series of experiments, which would give any reliable data, because of our ignorance of the individual conditions which obtain in each case. The factors which control the final result are so numerous and so many of them are unknown, that any attempt to approximate to them is practically impossible. No attempt was made therefore to do this experimentally, but information on the subject was sought from grain dealers and others and the question will be dealt with in another place.

Tribolium castaneum Hbst.

Plate IV.

As has been previously stated, this insect is not an active agent in the destruction of good wheat but lives on grains damaged by other insects and in the dust or frass produced by their attacks, and in reality it is a flour beetle. Lefroy[3] (1906) mentions it as "feeding upon grain, biscuits, and having a great liking for dried insects" and calls it the red grain beetle. The same author records it again[4] (1909) as "reared from wheat grains, wheat flour and oat meal as well as dried insects," but no details of its life-history are given. Fletcher[5] (1914) gives some details of its life-history and gives it the status of "a serious pest of stored products not only by the actual quantity eaten but on account of the extremely nauseous smell and taste which it communicates to infected substances."

[1] *Loc. cit.*
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] *Loc. cit.*
[5] *Loc. cit.*

PLATE IV.

Tribolium castaneum.

Fig. 1. The egg (× 13).
" 2. The freshly emerged larva (× 13).
" 3. The fully grown larva (× 10).
" 4. The pupa. Ventral view (× 13).
" 5. The adult (× 13).

Lathetius oryzæ.

" 6. The egg (× 13).
" 7. The freshly emerged larva (× 13).
" 8. The fully grown larva (× 10).
" 9. The pupa. Ventral view (× 13).
" 10. The adult (× 13).

Læmophlœus sp.

" 11. The egg (× 13).
" 12. The freshly emerged larva (× 13).
" 13. The fully grown larva (× 10).
" 14. The pupa. Ventral view (× 13).
" 15. The adult (× 13).

The small figures by the side of the larger ones indicate the natural sizes of the insects.

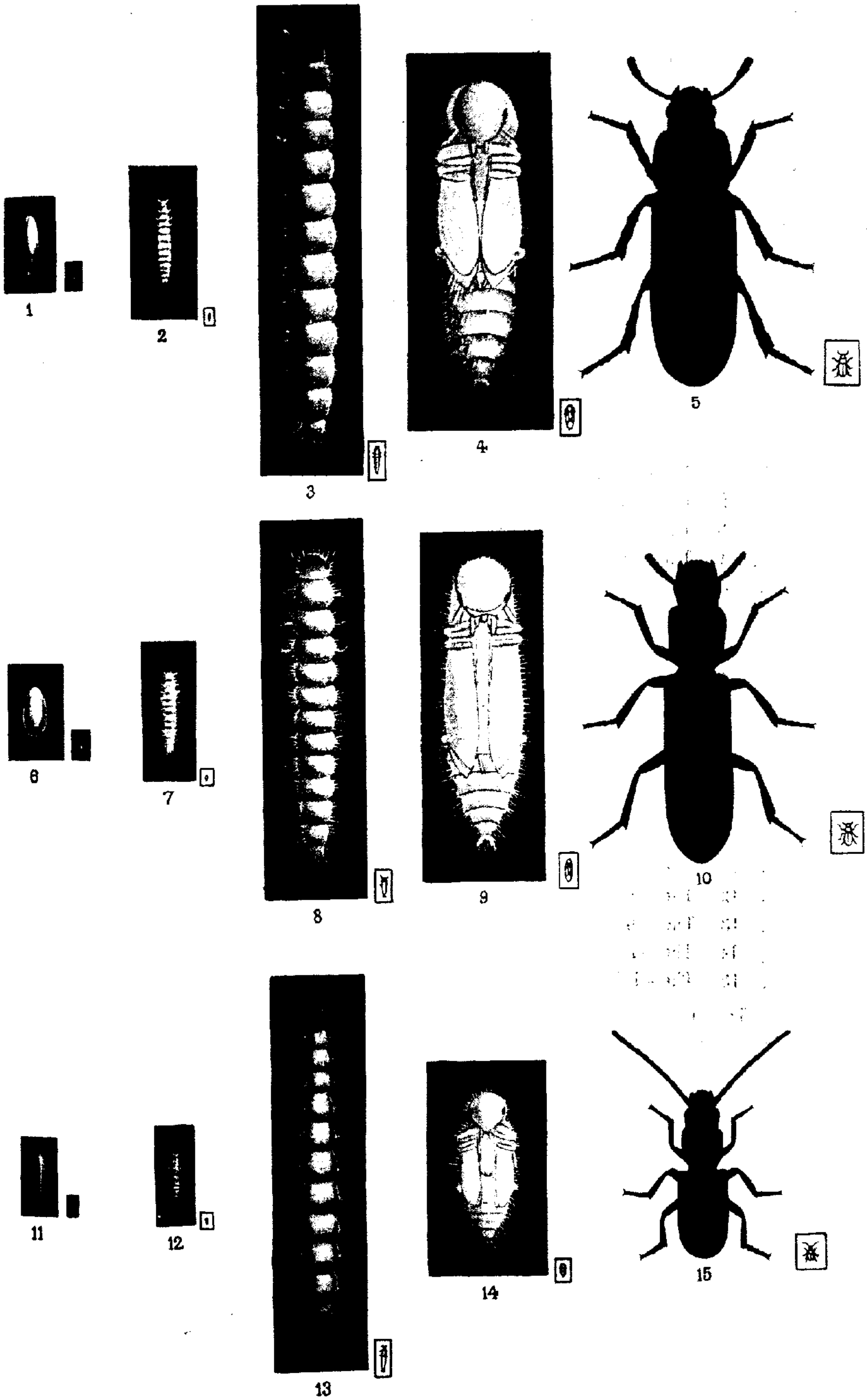
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PLATE IV.

Tribolium castaneum.

Fig. 1. The egg (×13).
,, 2. The freshly emerged larva (×13).
,, 3. The fully grown larva (×10).
,, 4. The pupa. Ventral view (×13).
,, 5. The adult (×13).

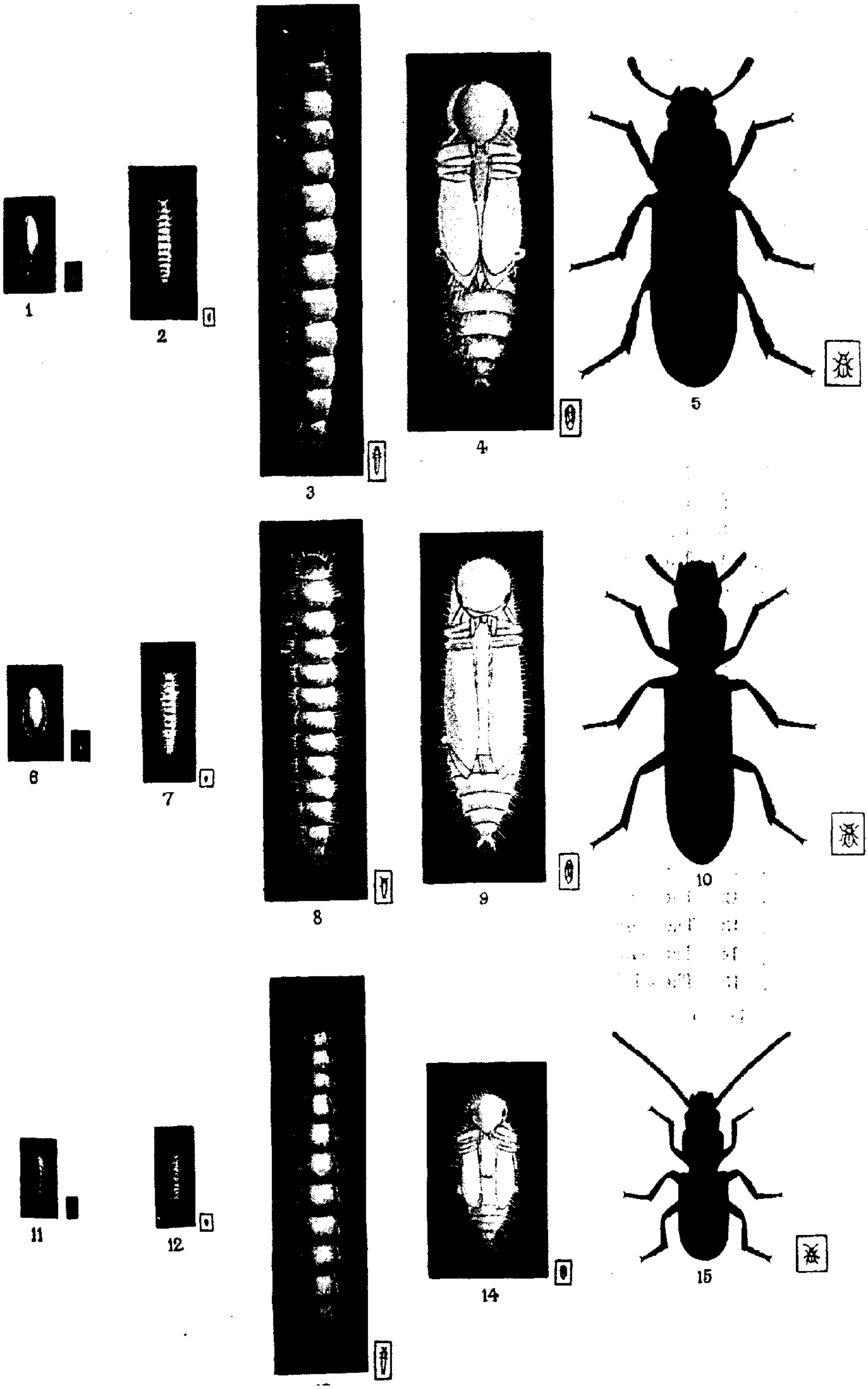
Lathetícus oryzæ.

,, 6. The egg (×13).
,, 7. The freshly emerged larva (×13).
,, 8. The fully grown larva (×10).
,, 9. The pupa. Ventral view (×13).
,, 10. The adult (×13).

Lœmophlœus sp.

,, 11. The egg (×13).
,, 12. The freshly emerged larva (×13).
,, 13. The fully grown larva (×10).
,, 14. The pupa. Ventral view (×13).
,, 15. The adult (×13).

The small figures by the side of the larger ones indicate the natural sizes of the insects.



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The egg. The egg is small, slender, cylindrical in shape, rounded at both ends and of whitish colour. The eggs are laid singly among the dust and floury matter in which the insects live and as they are sticky when they are laid they become covered with small particles of dust and flour which adhere to the surface. This renders the eggs very difficult to find.

The number of eggs laid by a female was not determined, but this is probably considerable judging by the rate at which this insect will increase under favourable conditions.

The larva. The freshly-emerged larva is about 1·0 mm. long, yellowish-white, slender, cylindrical, the thoracic segments narrower than the abdominal ones. The terminal segment is furnished with a pair of spine-like appendages, and all the segments have on them a number of fine hairs. When freshly emerged, the larva is beautifully translucent as if it were made of glass. After a while it becomes opaque with the ingestion of food. The most remarkable thing about the development of the larva is the short period between the successive moults and the number of them. The first moult often occurs the day following the emergence of the larva, and in the earlier stages the moults may succeed one another at intervals of two days. In the later stages a little longer period intervenes. The following table gives two examples of this :—

Date of hatching	Date of first moult	Date of second moult	Date of third moult	Date of fourth moult	Date of fifth moult	Date of sixth moult	Date of seventh moult
18 Aug. 14	19 Aug. 14	22 Aug. 14	24 Aug. 14	26 Aug. 14	28 Aug. 14	1 Sept. 14	5 Sept. 14
23 Sept. 14	24 Sept. 14	27 Sept. 14	30 Sept. 14	3 Oct. 14	7 Oct. 14	11 Oct. 14	19 Oct. 14

The usual number of moults is seven as recorded above, but variations have been noticed, eight moults being recorded in one case, and six in another.

As development progresses the colour of the larva changes until in the later stages it assumes a reddish-yellow colour, the colour being particularly developed in the head and the appendages of the terminal segment.

The full-grown larva is 6·3 mm. long and is of a general reddish-yellow colour. The head is much darker in colour and also the appendages of the last abdominal segment. The shape closely resembles that of the earlier stages. The eyes are distinct and the antennæ well developed. All the segments are furnished with fine hairs.

The pupa. This stage presents no striking features. The pupa is naked, yellowish-white, the dorsal surface covered with pale-coloured hairs and the

terminal segment with two spine-like processes resembling those possessed by the larva except that they are not pigmented.

The adult. The mature insect is a small reddish-brown beetle 3·5 mm. long. The head, thorax, and abdomen are distinct and the antennæ well developed.

The insect is very common in the wheat stores in the Punjab, sometimes being present in extraordinary numbers in wheat which has been attacked by other insects. This is especially so when the wheat has been damaged by *C. oryzæ* or *R. dominica*, both these insects producing a large quantity of dust and frass upon which the insect can live, but as has been previously pointed out this insect does not damage good wheat.

The length of life-history. This is apparently short under favourable conditions, the shortest period observed being 26 days (August-September). Considerable variation was noticed and in one case the period was extended to 59 days (August—October).

Latheticus oryzæ Waterh.

PLATE IV.

Although the specimens from which the original description of this insect was made came from Calcutta (Chittenden 1911), little or no attention has been given to it by Indian observers. Lefroy does not mention it in either of his books, and Fletcher[1] (1914) includes it in a list of insects attacking stored produce, but gives no details regarding it. It occurs fairly commonly in wheat stores in the Punjab, and a number of places in which it has been found are recorded on page 168.

The egg. The egg is shorter and broader than that of *T. castaneum* and ovoid rather than cylindrical in shape. It is opaque and the surface is smooth.

The larva. The freshly-emerged larva is very similar to that of *T. castaneum*, in fact in this stage it is very difficult to distinguish between them. The general colour is a yellowish-white with a slightly darker pigment on the head and appendages.

The full-grown larva, however, is easily distinguishable from that of *T. castaneum* being slightly shorter in length and lighter in colour, especially the head and appendages. The spine-like appendages to the last segment are also different in shape and lighter in colour. The segments are furnished with numerous light-coloured hairs.

[1] *Loc. cit.*

The number of moults passed through by the larva was found to vary from six to nine. The earlier moults followed in rapid succession, but the period between the later moults became gradually more extended.

The pupa. This stage is easily distinguishable from the corresponding stage of *T. castaneum*. The pupa is on the whole more slender, the thorax is not so clearly differentiated from the abdomen, and the wing cases are larger. In other respects the two stages are very similar.

The adult. Although closely resembling *T. castaneum* in many respects, yet careful examination reveals many differences between the two beetles. Chittenden[1] (1911) characterizes the species as :—" General form of *T. ferrugineum*, F., but rather narrower and the head relatively longer and broader and more square in general outline. Forehead and the middle of the epistoma gently convex, the former not very thickly but very distinctly punctured, about twice as broad as long, obliquely (but not much) narrowed anteriorly, declivous in front, impressed at the sides, emarginate in front, the ocular canthus not much encroaching upon the eyes. Antennæ rather short, thickest at the eighth joint, so that their general outline is somewhat fusiform. Thorax very little broader than the head across the eyes, a little narrower behind, very distinctly but not very thickly punctured : the angles obtuse, the sides somewhat straight very finely margined. Elytra as wide as the broadest part of the thorax, parallel, their surface somewhat uneven or wrinkled, each elytron with four or five scarcely impressed lines, with somewhat large punctures, the lines somewhat irregular or here and there interrupted, legs rather slender (Waterhouse)."

To this description no additions are necessary.

Length of life-history. This has only been worked out in a general way as the insect is not an important one. The shortest period observed was 25 days (August-September 1914), but during the same period the length of life-history was as extended as 39 days. The larvæ throve better on ordinary flour than on the floury frass produced by other insects.

Lœmophlœus sp.

PLATE IV.

The specimens of the insect found in the Punjab are apparently different from those of *L. pusillus* and its identification is still uncertain.

The egg. The egg is small, slender, cylindrical in shape and rounded at both ends. In colour it is a beautiful translucent white. The length is 0·6 mm.

[1] *Loc. cit.*

The larva. The freshly-emerged larva is about 0·7 mm. long, yellowish white, the head, and the spine-like appendages, on the anal segment, reddish brown. The larva is cylindrical in shape tapering a little to each end.

The full-grown larva, however, is different in shape. The head and thorax are narrow, gradually widening posteriorly, but the segments of the abdomen are markedly longer and broader, becoming progressively so until about the fourth segment which is the broadest, and then tapering away again to the terminal segment which is furnished with two pigmented spine-like appendages. The segments show a gradation in colour along the edges which gives them a margined appearance. Each of the abdominal segments is furnished with a few fine hairs. The general colour of the larva is a creamy-white with the exception of the terminal segment which is a reddish-brown. The full-grown larva is 4 mm. long and 0·5 mm. across the widest part of the abdomen.

In the course of its development the larva casts its skin four times, the last moult revealing the pupa.

The pupa. This is a curiously contracted, angular looking stage, and is small compared with the larva. It is creamy-white and is about 1·75 mm. long. Beyond its rather remarkable appearance it has no other noticeable characteristic.

The adult. The mature insect is a small flattened beetle about 2·0 mm. long. The general colour is a fuscus brown but the head and thorax are darker in colour, the antennæ are filiform.

The length of life-history. From the few examples reared through, there was found to be a good deal of variation in the period occupied by the insect in completing its development. The shortest period noticed was 36 days (September-October 1914), but even during this period of the year the number of days was as many as 75 in one case.

The adult beetles are sometimes capable of living for very extended periods. For example, a male and a female beetle were found copulating on 16th March 1914 and were isolated. One beetle lived until 21st May, and the other died only on 10th November. During this time the insects had been fed on flour, and although soon after they were isolated a number of larvæ were seen, these apparently did not mature. The females apparently pair with the males several times during their existence. A male and female, found copulating, were isolated on 17th March 1914. On 4th April they were observed to be again coupling, and this was also noticed on the following

dates :—5th, 6th, 7th, and 17th May and 11th June. The male died on 29th June and the female on 10th August.

Sitotroga Cerealella Oliv.

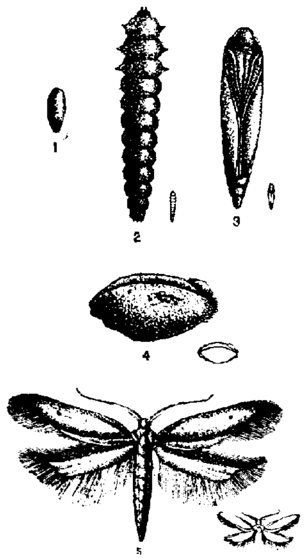


FIG. 1.

This small moth was found fairly commonly in the Gurdaspur district, but does not seem to be important in other parts of the Province.

The egg. This is small, pinkish in colour, about 0·5 mm. long, ovoid in shape, one end being wider than the other, and both ends are rounded. The surface of the egg-shell is striated longitudinally. The number of eggs laid by each female is about one hundred. Of three females kept under observation one laid 118 eggs, another 87 eggs and the third 80. The eggs are laid in clusters in any convenient position, often in the groove in the grain or in holes in the grains made by other insects.

The larva. The freshly-emerged larvæ are very small and extremely active. They crawl about the grains in search of a suitable one to attack, and generally effect an entrance through a crack or abrasion in the pericarp.

Having once entered the grain the larva remains inside it for the remainder of its development.

The full-grown larva is a small grub or caterpillar about 5·0 mm. long. The head is small, light brown or yellowish-brown in colour. The thoracic segments are furnished with three pairs of true legs, and there are also four pairs of prolegs, one pair on each of the third to sixth abdominal segments.

When the larva is ready to pupate it extends the cavity which it has excavated to the outside of the grain, and a small hole is gnawed out on the surface of the grain. The larva then spins a thin cocoon of silken material, and one end of this cocoon projects through the hole made in the surface of the grain, giving the appearance of a small blister projecting from the surface. The larva then pupates inside the cocoon. This piercing of the side of the grain is a provision to enable the moth, when it emerges from the pupa, to leave the grain easily, for the moth itself is much too delicate to force or make its way through the tissues of the grain without some provision being made for it.

The pupa. This stage is a typical lepidopterous pupa, and offers no special points for remark.

The adult. The mature insect is a small moth 6·25 mm., from tip to tip of the wings, which are delicate and heavily fringed with hairs on the posterior margin. The antennæ are long and filiform, practically free from hairs, and the labial palps are long and project anteriorly from the head.

The moth emerging from the pupa breaks through the silken cocoon where it projects beyond the surface of the grain, and creeps out. It then gradually makes its way up between the grains to the surface of the wheat where it pairs. The female then lays her eggs and dies. A very efficient way therefore of preventing the increase in numbers of this insect, would be either to fill the receptacles containing the wheat right to the top, so that no space would be left at the top for the insects to pair in, or to produce the same state of affairs by covering the wheat with gunny bags or *chittais* and fill up the space with *bhusa* (broken wheat straw).

The length of life-history. In the period during which this insect was kept under observation, the length of life-history was found to vary from 30 days (9th September—8th October 1914) to 142 days (12th October 1914 to 2nd March 1915). This latter extended period was perhaps exceptional and the larva was probably in a state of hibernation during the greater part of it, for several moths emerged after a period of between 50 and 60 days, the eggs

for all of them having been laid on the same date, *viz.*, 12th October 1914. Between September and December the length of life-history gradually increases from 30 days to about 65 days and then the larva probably goes into hibernation.

PARASITES.

In examining wheat which was badly attacked by *R. dominica* and *C. oryzæ* a number of tiny black Chalcid parasites was often seen. In cases where large numbers of these two beetles were being reared for experimental purposes, the parasite was often noticed. No appreciable diminution in the number of the beetles was, however, ever observed that could be attributed to the activities of the parasite, and from general observations in wheat stores its effect as an efficient check upon the multiplication of the beetles never appeared marked. In any case, the parasite could not be used as a practical means of keeping the wheat free from the attack of the beetles, so that no further observations were made upon it. No hymenopterous parasite was ever observed attacking *A. undulatus*.

GENERAL REMARKS.

From the records which have been given above it will be seen that the three insects, *A. undulatus*, *R. dominica* and *C. oryzæ*, which are the most important agents in causing damage to stored wheat in the Punjab, offer many differences as well as resemblances, and the grain always seems to be liable to the attack of one or other of them, no matter under what conditions it is stored. As has been mentioned previously, the relative importance of the three insects varies from place to place depending very largely upon the humidity of the place in question. It is very difficult to say therefore which is the most important insect of the three, or which is responsible for the greatest loss, but of the three it may be said that *A. undulatus* seems to possess the greatest capacity for withstanding adverse conditions. It is handicapped however from the point of view of rapid multiplication by the comparatively small number of eggs laid by the female, and in this respect *R. dominica* and *C. oryzæ* possess a distinct advantage. In general, therefore, the attack of *A. undulatus* is as a rule continuous and steady while that of *C. oryzæ*, and probably *R. dominica* also, is somewhat discontinuous, depending upon whether conditions are favourable or not, but when things are satisfactory, they can do a great deal of damage in a short time. This bears out the experience of grain dealers who say that when grain becomes attacked by *susri* (meaning *C. oryzæ* and *R. dominica*) then a great deal of damage is effected in a short time, but that *khapra* (*A. undulatus*) works more slowly.

How infection takes place. The question often arises how stored grain becomes infected. As far as the conditions under which the ordinary grain dealer stores his wheat are concerned, infection invariably results from the storehouses and godowns containing numbers of insects when the wheat is put into them. It has been pointed out above, how the larvæ of *A. undulatus* hibernate in the cracks and crevices in the walls of storehouses and the same thing applies to the adults of *C. oryzæ* and *R. dominica*. It is therefore no matter for surprise, when the perfunctory cleaning process to which the grain stores are usually subjected is considered, that the newly-stored wheat becomes infected at once. This is probably the most important source of infection under the methods of storing wheat at present in vogue in the Punjab, for it is almost impossible to conceive of a building which would offer more convenient lurking places for insects, than the ordinary godown or *kotha* with its walls of imperfect masonry, often covered with mud plaster full of cracks and crevices, and the general indifferent state of repair in which the places are kept.

Apart from these considerations the grain is probably open to infection in other ways. All three insects possess wings in the adult state and *R. dominica* has often been caught flying, and though the other two have not, yet there does not seem to be any reason why they should not. None of the insects, however, have been found attacking wheat in the field or on the threshing floor, though here again, considering the time wheat often lies about in the open before it is stored, it is not impossible that adults may visit it and deposit eggs among the grains. Samples of wheat from threshing floors have not, on examination, confirmed this, and it is probable that the fact that the heaps of grain are usually left in the sun, would prohibit the advent of the beetles, as these insects are essentially inhabitants of houses and store places and not outdoor insects. In any case it is certain that in the Punjab where wheat godowns are often built in rows, insects pass from one chamber to another and extend their depredations in that way.

THE EXTENT OF THE DAMAGE CAUSED BY THE INSECTS.

It has been stated above that no experiments were carried out to test the amount of damage caused by these three insects owing to the practical difficulty of reproducing the extremely varied conditions under which the insects live in nature, and unless this is done the figures obtained are of little practical value. Enquiries were made, however, from grain dealers in the

various places visited, as to the amount which they reckoned to write off each year as loss due to insect attack. The replies, as expected, were varied, some being as small as two per cent. and others as much as five or seven per cent., and the general impression was that the average loss per annum was about two and-a-half per cent. All were practically unanimous in saying that *susri* (*C. oryzæ* and *R. dominica*) when it occurred, produced more loss than *khapra* (*A. undulatus*), but as has been pointed out *A. undulatus* works more continuously than the other two and is perhaps responsible for more general damage than the others. That it is sometimes responsible for considerable damage is shown by some figures supplied by Rai Sahib Sewak Ram of Gangapur, who during the season 1914-15 lost 300 maunds of wheat out of 5,000 maunds, chiefly owing to the depredation of *A. undulatus*.

CHAPTER II.

First Series of Chemical Experiments.

In 1911 some experiments were started at Lyallpur to ascertain if any simple and easy method could be devised for immunizing stored grain against the attacks of insect pests of the "weevil" type.

Such method must *a priori* aim at simplicity, cheapness, and freedom from danger, and must leave the grain in an undamaged condition both as a food and for seed purposes. Since these insects are found for the most part in mud huts or other similar dirty places used as granaries by the Punjab villager, one of the first tests made was the substitution of a cheap galvanized iron bin for the common store which is usually only the earthen floor of a mud hut. In order to keep down the cost of construction the bin was made of thin galvanized iron (·022" thick No. 24 B.W.G.). This runs about 106 square feet to the cwt. and costs at Lyallpur Rs. 13/- per cwt. They were made cylindrical in shape; one bin being 8 feet deep with a diameter of 5½ feet and the other 7½ ft. deep with a diameter of 6½ feet. Both were supported on the outside by a roughly made mud wall about twelve inches thick. The bins were roofed over with iron sheets to prevent the rain water getting in, but as the roof did not project over the walls to a sufficient extent the water was blown in under the eaves during the monsoon rains in the first season. This defect was afterwards rectified.

One of the principal objects of these bins was to test the lethal effect of carbon dioxide gas on the weevils and so all joints were soldered after the edges of the plates had been riveted in order to prevent the gas from leaking. Cole[1] experimenting with *C. oryzæ* and *C. granaria* showed that these insects remained alive for several days in moist air containing 80% of carbon dioxide, but that the pure dry gas was very fatal, acting either as a poison or as a desiccating agent or both. Cole regards moisture as the important factor.

Lefroy[2] considers that sun-dried wheat to which moisture does not get access is immune to insect attack provided it contains 7% of moisture or less.

[1] Cole. *Journal of Economic Biology*, I, 63—71.

[2] Private communication by letter, September 1912.

We shall show later that this opinion appears to be based upon insufficient information both biological and physical, since he refers only to *C. oryzæ*.

The wheat harvest in the Punjab falls in the months of April and May, usually commencing about the middle of April. The harvest is threshed during the dry hot month of May when the temperature of the air is frequently higher than 115°F in the shade and 160°F by the black bulb thermometer in vacuo.

The following table shows the moisture contents of average samples of wheat harvested and threshed under ordinary country conditions at Lyallpur :—

TABLE XVII.

Showing the amount of moisture present in freshly harvested wheat in the Punjab (Lyallpur), June, 1914.

		Hard wheat	Soft wheat
Moisture lost at 70°C.	in 4 days ...	2·03%	1·76%
	in 18 days ...	4·6%	3·4%
Moisture lost at 100°C.	in 1 day ...	8·7%	8·6%
	in 12 days ...	8·7%	8·8%

Such wheat as this then practically complies with the conditions suggested by Lefroy as requisite for immunity against insect attack. It might be thought that wheat takes up moisture in some quantity during the succeeding moist months of July and August, when the monsoon is in progress. To test this, samples of both hard and soft wheat were purchased from week to week during the season, in the Lyallpur market. These samples represent wheat grown in the preceding season, threshed in May, and stored either in the Lyallpur market or in the villages of the surrounding district. From the number of samples taken we may assume them to be truly representative of the stored grain found in this tract during the months of July to November. Tables XVIII and XIX show that the moisture content of the wheat has not advanced to any considerable extent over that of the recently harvested grain in May, the wheat still remaining in what we should ordinarily term a " dry " condition.

TABLE XVIII.

Showing the amount of moisture present in bazaar (Lyallpur) wheat during the months of July, August, September, October and November 1914.

Variety . . . Soft bazaar wheat.

Date of purchase of the sample	Date of experiment	MOISTURE % AT 70°C.			MOISTURE % AT 100°C.		
		Loss % in 24 hours	Further loss %	Total moisture %	Loss % in 24 hours	Further loss %	Total moisture %
2-7-1914	21-9-1914 to 8-10-1914	4·52	1·97	6·49	7·32	1·85	9·17
8-7-1914	Do.	5·92	1·50	7·42	8·32	1·42	9·74
13-7-1914	Do.	4·95	1·99	6·94	7·34	1·81	9·15
18-7-1914	Do.	6·05	1·50	7·55	8·37	1·55	10·92
28-7-1914	Do.	5·90	1·60	7·50	8·23	1·72	9·95
19-8-1914	Do.	5·91	1·70	7·61	7·99	2·03	10·02
29-8-1914	Do.	6·76	1·96	8·72	9·76	1·29	11·05
12-9-1914	Do.	5·32	2·00	7·32	8·02	1·81	9·83
15-9-1914	5-11-1914 to 25-11-1914	3·99	1·60	5·59	7·17	0·69	7·86
30-9-1914	2-12-1914 to 15-12-1914	4·89	2·58	7·47	8·41	0·79	9·20
7-10-1914	5-11-1914 to 25-11-1914	5·49	2·08	7·57	9·31	0·79	10·10
17-10-1914	Do.	6·17	1·90	8·07	9·25	0·86	10·11
23-10-1914	23-11-1914 to 15-12-1914	5·53	2·27	7·80	9·34	0·42	9·76
29-10-1914	Do.	5·97	2·18	8·15	9·88	0·16	10·04
7-11-1914	Do.	6·08	2·35	8·43	9·61	0·53	10·14
12-11-1914	Do.	5·87	2·10	7·97	9·48	0·54	10·02

TABLE XVIII—(*continued*).

Showing the amount of moisture present in bazaar (Lyallpur) wheat during the months of July, August, September, October and November 1914.

Variety . . . Soft bazaar wheat.

Date of purchase of the sample	Date of experiment	Germination value of fresh sample	Germination value after heating the sample at 70°C.		Germination value after heating the sample at 100°C.	
			For 24 hours	At the close of experiment	For 24 hours	At the close of experiment
2-7-1914	21-9-1914 to 8-10-1914	99	99	96	*Nil*	*Nil*
8-7-1914	Do	100	96	98	*Nil*	*Nil*
13-7-1914	Do.	99	98	94	*Nil*	*Nil*
18-7-1914	Do.	92	94	86	*Nil*	*Nil*
28-7-1914	Do.	98	100	88	*Nil*	*Nil*
19-8-1914	Do.	96	93	96	*Nil*	*Nil*
29-8-1914	Do.	95	90	90	*Nil*	*Nil*
12-9-1914	Do.	98	99	89	*Nil*	*Nil*
15-9-1914	5-11-1914 to 25-11-1914	99	98	99	*Nil*	*Nil*
30-9-1914	2-12-1914 to 15-12-1914	98	92	92	*Nil*	*Nil*
7-10-1914	5-11-1914 to 25-11-1914	91	94	74	*Nil*	*Nil*
17-10-1914	Do.	89	93	86	*Nil*	*Nil*
23-10-1914	23-11-1914 to 15-12-1914	92	95	90	*Nil*	*Nil*
29-10-1914	Do.	75	86	80	*Nil*	*Nil*
7-11-1914	Do.	28	25	18	*Nil*	*Nil*
12-11-1914	Do.	75	72	45	*Nil*	*Nil*

TABLE XIX.

Showing the amount of moisture present in bazaar (Lyallpur) wheat during the months of July, August, September, October and November 1914.

Variety . . . Hard bazaar wheat.

Date of purchase of the sample	Date of experiment	MOISTURE % AT 70°C.			MOISTURE % AT 100°C.		
		Loss % in 24 hours	Further loss %	Total moisture %	Loss % in 24 hours	Further loss %	Total moisture%
2-7-1914	15-10-1914 to 29-10-1914	3·10	2·99	6·09	7·68	0·90	8·58
8-7-1914	Do.	2·78	2·83	5·61	7·08	1·09	8·17
13-7-1914	Do	4·34	2·90	7·24	8·66	1·20	9·86
18-7-1914	Do	3·60	2·85	6·45	7·64	1·02	8·66
28-7-1914	Do.	5·20	2·65	7·85	8·86	1·24	10·10
19-8-1914	Do	4·24	2·82	7·06	8·25	1·10	9·35
29-8-1914	Do	4·08	2·40	6·48	7·73	1·22	8·95
12-9-1914	Do.	5·16	2·72	7·88	9·22	1·30	10·52
15-9-1914	5-11-1914 to 25-11-1914	4·66	2·00	6·66	7·92	0·94	8·86
30-9-1914	Do.	4·46	1·36	5·82	7·40	0·93	8·33
7-10-1914	Do	4·84	1·84	6·68	7·78	0·96	8·74
17-10-1914	Do	5·20	1·86	7·06	8·85	0·91	9·76
23-10-1914	23-11-1914 to 15-12-1914	4·54	2·50	7·04	8·73	0·08	8·81
29-10-1914	Do	6·15	1·90	8·05	9·96	0·66	10·62
7-11-1914	Do.	5·38	2·10	7·48	8·88	0·48	9·36
12-11-1914	Do.	5·63	2·72	8·35	9·48	0·48	9·96

TABLE XIX—(*continued*).

Showing the amount of moisture present in bazaar (Lyallpur) wheat during the months of July, August, September, October and November 1914.

Variety......Hard bazaar wheat.

Date of purchase of the sample	Date of experiment	Germination value of frseh sample	GERMINATION VALUE AFTER HEATING SAMPLE AT 70°C.		GERMINATION VALUE AFTER HEATING SAMPLE AT 100°C.	
			After heating for 24 hours	At the close of experiment	After heating for 24 hours	At the close of experiment
2-7-1914	15-10-1914 to 29-10-1914	99	99	100	*Nil*	*Nil*
8-7-1914	Do.	99	99	98	*Nil*	*Nil*
13-7-1914	Do	100	100	98	*Nil*	*Nil*
18-7-1914	Do.	99	99	97	*Nil*	*Nil*
28-7-1914	Do	96	94	88	*Nil*	*Nil*
19-8-1914	Do.	88	82	82	*Nil*	*Nil*
29-8-1914	Do.	95	99	97	*Nil*	*Nil*
12-9-1914	Do	96	87	92	*Nil*	*Nil*
15-9-1914	5-11-1914 to 25-11-1914	94	91	87	2	*Nil*
30-9-1914	Do.	99	100	96	10	*Nil*
7-10-1914	Do.	97	95	95	4	*Nil*
17-10-1914	Do.	100	98	88	*Nil*	*Nil*
28-10-1914	23-11-1914 to 15-12-1914	96	92	87	*Nil*	*Nil*
29-10-1914	Do.	99	100	97	*Nil*	*Nil*
7-11-1914	Do.	91	96	92	*Nil*	*Nil*
12-11-1914	Do.	93	94	92	*Nil*	*Nil*

The grain was hard and there was no sign of germination or mould. It appears questionable if wheat is capable of taking up moisture from a moist atmosphere under ordinary conditions of storage.

Physically speaking it consists of bad heat-conducting material and consequently it is unlikely to undergo the rapid temperature changes necessary to induce the condensation of moisture from a moist atmosphere, for low conductivity also means that it is a poor radiator of heat. During germination the grain appears to take up water only in the liquid state, for in experiments conducted by one of us to determine the amount of carbon dioxide given off during germination no germination took place unless the grain was in contact with a water film. In this way the grain is protected against germination under unsuitable conditions. The slight increase in moisture during the month of July and subsequent damp periods is probably surface moisture and insufficient to be taken up by the grain substances. This appears to be borne out by the figures of Tables XVII and XVIII which show that more moisture is lost at 70°C during the first twenty-four hours than is the case in the freshly harvested wheat.

The case is not the same as starch itself, though this is one of the principal ingredients of wheat, and notwithstanding that commercial starches are very hygroscopic and contain from 18% of water in the case of ordinary commercial starches[1] to 20·4% in the case of air-dried potato starch.[2]

In the wheat grain the starch is enclosed in a cellular envelope which evidently does not allow of the ready access or loss of moisture in the form of vapour. The same probably applies to the protein and other chemical contents of the endosperm. We conclude therefore that ordinarily speaking wheat is not a hygroscopic substance and cannot take up much more moisture than it contains at harvest time, the time when the wheat is at its driest and that "*moisture in the wheat*" is not the important factor in insect attack. Our results for the moisture content of wheat do not agree with those quoted by Fletcher.[3] This author makes no mention of the conditions under which the moisture determinations were made.

2. Early experiments with carbon dioxide.

In selecting a germicide for use in India on such a common article of food as wheat, the range of chemical substances at our disposal is considerably narrowed by the cheap and ignorant labour which will be employed in its

[1] Wiley. *Agricultural Analysis*. Vol. III, p. 368.

[2] Davis and Daish. *Jour. Agri. Science*, Vol. VI, Part II, p. 165.

[3] Fletcher, T. Bainbrigge. Weevil and Dry Wheat. *Agri. Jour. India*, Vol. VI, Part IV, October 1911, pp. 340—341.

application. Even in a large and modern wheat store like an elevator we in India cannot eliminate the Indian coolie and it is more than likely that the use of such substances as carbon disulphide, benzene, hydrocyanic acid, will raise the insurance rates to a prohibitive figure, for their use in this country would sooner or later be attended with serious accidents. The possibility of their use by the Indian villager in his granary is even more remote. We therefore differ from Lefroy when he says that carbon disulphide can be used in the new elevator to be erected at Lyallpur. In view of this and as Cole's results were by no means conclusive or exhaustive, it was thought advisable to test once more the effect of carbon dioxide, as this gas if it could be rendered effective would be cheap and easy of application and practically free from danger in an elevator. At the same time we decided to take records of its effect upon the germinating value of the grain.

Badcock[1] states that corn (*Zea Mays*) stored for thirty days in a sealed flask with carbon dioxide failed to germinate at the end of this period, so the process to be successful would have to be applied either for a sufficiently short period in the case of strong admixtures of carbon dioxide, or for longer periods for the gas in a more diluted form, but in all cases for such a time as will leave the germinating power unimpaired.

The gases of stored wheat and the effect of carbon dioxide on the germinating power of the grain. The amount of carbon dioxide given out by stored wheat.

Wheat like most seeds gives out carbon dioxide when in the dormant condition. Since the seed is dry during this period the carbon dioxide cannot result from the combustion of the carbohydrates of the endosperm, for these only become available as a food after the hydrolysing action of such enzymes as amylase and cytase which are only active after the grain is thoroughly moistened and at a suitable temperature. It must result therefore from the gradual decay or combustion of material in the embryo itself. This is borne out by the observation that in old stored grain the embryo is found to be much shrunk while the food materials of the endosperm are unimpaired. Indeed very often the grain has lost the power of germination by reason of this death of the embryo though it may still serve as a perfectly good food. As soon as germination sets in, the evolution of carbon dioxide rapidly increases through the combustion of a large portion of the carbohydrates of the endosperm.

The bins mentioned above were charged with wheat on 12th June 1911, and the air in one bin displaced with carbon dioxide on the 1st of August.

[1] Badcock. *Wisconsin Station Research Bull.* 22, pp. 87—181.

Samples of the bin gases were withdrawn on 4th April 1912, and their analysis gave the following figures :—

TABLE XX.

Analysis of gases from wheat bins, 1911-12.

	Bin (A). No carbon dioxide added	Bin (B). Carbon dioxide added
Carbon dioxide per cent. in the bin gases	17·5 19·1 19·0 19·2	16·5, 16·3 Top; 16·7 Middle; 16·8, 16·4 Bottom } of the bin.
Oxygen per cent. in the bin gases.	3·6 3·4 3·4	5·2 Top; 5·7, 5·2, 4·7 Middle; 4·3 Bottom } of the bin.
Germinating power of a sample from the middle of the bin.	97%	93%

On opening the bins and extracting the wheat, it was found that the grain in (A) had become wet through the monsoon rains having blown in under the edges of the roof, and as the bins had been constructed to be water-tight at the bottom and sides, this water could not drain away and was consequently absorbed by the wheat, inducing partial germination in about 1½ feet of grain at the bottom.

The grain was mouldy and we account for the high percentage of carbon dioxide in this bin as derived from the germination of the grain, for in the following year the bins were again filled with wheat and the roofs reconstructed so as to render them waterproof against wind-driven rain and the following table shows the analysis of the gases obtained :—

TABLE XXI.

Showing the analysis of wheat bin gases, 1912-13.

	Bin (A). No carbon dioxide added	Bin (B). No carbon dioxide added
Carbon dioxide per cent in the bin gases	5·96, 5·84 } bottom of the bin.	4·27, 4·36 } half way down the bin. 4·58, 4·64 } bottom of the bin.
Germinating power ...	96%	98%

The oxygen contents of these bins A and B in the 1911-12 experiment also show that in the case of A some 78% of the oxygen originally present in the bin gases has been "consumed" and in bin B not less than 71%. In 1912-13 unfortunately no record was taken of the amount of oxygen present at the end of the experiment. It appears from these figures that in a bin of 190 cubic feet capacity and a depth of eight feet the gases of the bin after the wheat has been stored for eleven months contain only 5·9% carbon dioxide

and in the shorter bin of greater capacity having a depth of $7\frac{1}{2}$ feet and 248 cubic feet capacity, only 4·5% of carbon dioxide. This carbon dioxide is produced as a result of the respiration of the stored grain and is probably derived entirely or almost entirely from the combustion of nutrient substances of the embryo itself.

We do not know at what rate carbon dioxide is respired by stored grain, it probably differs with temperature and moisture conditions, increasing as the temperature rises and as the amount of moisture and available oxygen increases. Whatever has been the rate of carbon dioxide production, the rate of diffusion has evidently been sufficient to lower the percentage of this gas to about 4% under the conditions of the above experiment.

In Table XX bin B was charged with carbon dioxide by a pipe entering the tank at the bottom, using a cylinder of compressed gas and admitting the gas from it into the bin slowly so as to displace the air above. In this manner we displaced the whole of the air. But at the end of eleven months the bin gases only contained about 16·5% carbon dioxide so that diffusion has evidently not proceeded further than this within eleven months. In bin A of the same year carbon dioxide production was proceeding at a fairly high rate long after the date on which B had been charged with this gas due to the accidental introduction of water and the consequent wetting and partial germination of the grain.

In taking samples of gases from the bins in the above experiments, small copper side tubes having a bore of $1\frac{1}{2}$ mm. were soldered into the bins during its construction. These were attached to a bulb filled with mercury and the gases drawn in by allowing the mercury to flow into a second bulb. By means of a three-way tap the first three or four samples collected could be rejected without disconnecting the apparatus so ensuring a representative sample of the gas.

Amount of carbon dioxide given out by germinating wheat.

To obtain some idea of the amount of carbon dioxide respired during germination the following experiment was made:—

Into a tube (18 cm. × 4 cm.) were placed some glass beads, and 25 cc. of boiled and cooled water. Above the beads were placed 50 grams of wheat which had been washed with boiled and cooled water.

The tube was then stoppered with a rubber bung and completely immersed in the water of a constant temperature bath—at 32°C. Arrangements were made to admit pure air saturated with water vapour at laboratory temperature, the air being freed from carbon dioxide by soda lime and by

potash, and from spores, bacteria, etc., by passing through three Glover's towers packed with sterile cotton wool.

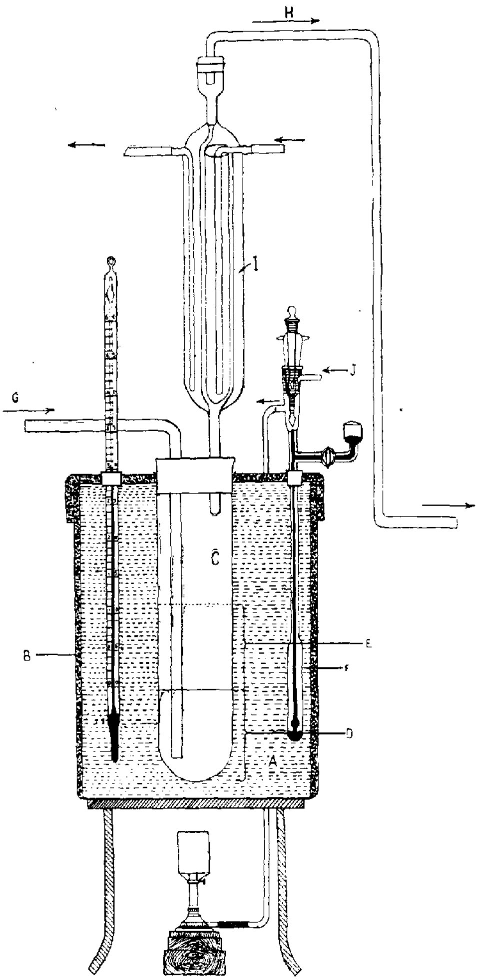


Fig. 2.

The gases issuing from the germination vessel pass upwards through a perpendicular condenser to ensure the return of any moisture tending to distil over from the tube when this is at a higher temperature than the laboratory.

Beyond this the gases are first dried and the carbon dioxide afterwards absorbed in potash bulbs and in soda lime tubes, using ordinary precautions.

Before commencing the experiment and after charging the tubes with wheat, this and the condenser were sterilized with chloroform vapour for 15 minutes, the vapour being afterwards removed by a rapid passage of sterile air. A similar experiment was made to determine the amount of carbon dioxide generated by the germinating grain *without the passage of air*, that is,

supplying the wheat with only the air present in the tube and aspirating the carbon dioxide out into the absorption tubes at the end of the experiment.

TABLE XXII.

Showing the number of grams of carbon dioxide evolved during the germination of 100 grams of wheat at a temperature of 32°C.

TUBE A.—MOIST STERILE AIR ASPIRATED THROUGH THE APPARATUS DURING THE EXPERIMENT			TUBE B.—NO AIR ASPIRATED THROUGH DURING GERMINATION	
Date	Period of growth in days	Grams of carbon dioxide	Period of growth in days	Grams of carbon dioxide
7-4-11	0	0	1	0·08
10-4-11	3	2·5	3	0·8
11-4-11	4	3·06	4	1·76
12-4-11	5	3·28	5	2·4
13-4-11	6	3·52	6	2·8
14-4-11	7	4·14	7	2·9
15-4-11	8	4·70	8	2·96
16-4-11	9	5·34	9	2·99
17-4-11	10	5·94	10	3·02
27-4-11	20	10·92	20	3·5
4-5-11	30	15·54	30	3·88
15-5-11	41	19·00	40	3·98
24-5-11	50	19·60	50	4·02
2-6-11	62	20·44	70	4·09
10-6-11	70	21·90	130	4·22
17-6-11	77	22·54	...	..
29-6-11	89	25·34	...	...
8-7-11	99	27·32	...	...
A duplicate of this experiment gave 34 grams of CO_2 in 60 days from 100 grams of wheat.				

Figure 2 gives a sketch of the apparatus used and Table XXII and the curves in Fig. 3 (p. 220) show the results obtained.

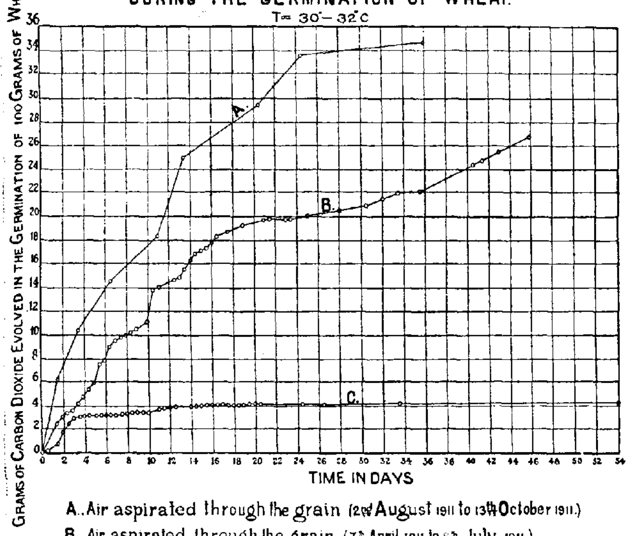
From these tables it will be seen that when the grain is supplied with plenty of air the amount of carbon dioxide produced varies from 27 to 34% of the weight of the grain, and when no air beyond that contained in the germination tube is given, the amount of carbon dioxide produced is only 4·2%. At the end of the experiment the contents of the tubes A and B were examined.

A was found to contain a blackened mass of husks and shrivelled sprouts. B had sprouted but oxidation had not proceeded far enough to produce discoloration. These observations are of some interest and importance and might with advantage be repeated and extended. They provide a partial explanation of the results of the first bin experiment where we found a high carbonic acid content in the bin gases of that vessel to which water had gained admission; evidently there was a sufficiency of both air and water to admit of extensive germination of the grain at the bottom of the bin and a consequent

production of some quantity of carbonic acid. They show in a very marked manner the importance of allowing for adequate soil aeration during germina-

Fig. 3.

AMOUNT OF CARBON DIOXIDE LIBERATED
DURING THE GERMINATION OF WHEAT.
T = 30°–32°C



A..Air aspirated through the grain (2nd August 1911 to 13th October 1911.)
B..Air aspirated through the grain (7th April 1911 to 8th July 1911)
C..Grain germinated in closed vessel no air aspirated through the grain (4th April 1911 to 19th July 1911)

tion—a point of considerable importance in the farming of canal-irrigated lands in the Punjab where the presence of a fine clay in the canal water tends to produce an intensely hard surface crust of an almost cement-like nature.

Wheat was again stored in these bins during 1913-1914 and the top surface of the grain protected with eight inches of *bhusa* (the broken straw obtained by threshing the corn by means of bullocks treading out the grain on a prepared piece of hard ground). In all cases the grain used for these experiments was that grown on the Government Farm at Lyallpur, and threshed out by the above method. It was, however, cleaner on the whole than ordinary country wheat when introduced into the chambers. Early in September 1913 one of us examined the contents of these bins and found them to contain a number of specimens of the larva of *A. undulatus* and also *Tribolium castaneum;* of these, only the former is an active agent in damaging wheat. It is clear therefore that Lefroy's statement cannot be applied to the problem

in this province, since this opinion is based upon experiment with *C. oryzæ* only, and as shown in Chapter IV the other insects, which damage wheat in the Punjab, do not behave in the same way towards moist and dry conditions as does *C. oryzæ*.

Simultaneously with the first experiment in bins, we made some observations on the effect of carbon dioxide on the germinating power of wheat in weevil-infected samples.

Experiment. Since the introduction of grain into a bottle containing a mixture of air and carbon dioxide without disturbing the composition of the gas, presents almost insurmountable experimental difficulties, the following method was adopted. This method we consider gives a reliable comparative figure, for though the analysis of the gases at the end of the experiment does not represent the amount of carbon dioxide present in the gaseous mixture at the start but the sum of this gas present in the original mixture plus that derived from the respiration of the grain and any weevils present. Using the same quantity of grain for all experiments and making time and temperature conditions equal—these two latter factors will cancel out and give us the comparative effect of the carbon dioxide added at the commencement, on the germinating power of the grain and on the weevils if the grain contains them.

(A) 50 grams of good wheat, the germinating power of which was first determined, were placed in a wide-mouthed and stoppered bottle of 150 cc. capacity. Carbon dioxide gas from a Kipp's apparatus, well washed to free it from acid vapours, was then allowed to flow in by a tube reaching to the bottom of the bottle, the air of the bottle being displaced upwards. The amount of gas was regulated by counting the number of bubbles passing through the last wash bottle. 24 bottles containing wheat were taken and quantities of carbon dioxide corresponding to 28, 56, 85, 115, 170, and 240 bubbles of the gas were added, so as to obtain different quantities of the gas in each bottle.

The bottles were closed by well greased stoppers immediately after the addition of the carbon dioxide. Three months after, the gases of the bottles were extracted over mercury and analysed, and the grain tested for its germinating power.

Table XXIII gives the results obtained and confirms Badcock's[1] statement that the presence of carbon dioxide does affect the germinating power of grain. Badcock's experiment was made on maize and he found this grain stored for 30 days in a sealed flask charged with the gas failed to germinate at the end of that period.

[1] *Loc. cit.*

From our experiments there does not appear to be any numerical relationship between the loss in germinating power and the amount of the gas to which the grain has been subjected.

TABLE XXIII.

The effect of varying quantities of carbon dioxide on the germinating power of wheat.

1	2	3	4	5	6	7	8
Date of charging the bottle with grain and CO	Date of analysis	Number of bubbles of CO_2 with which the bottle was charged	Quantity of wheat used	Bottle No.	Percentage of CO_2 found on analysis	Germination value at end of experiment	Germination value of the grain before experiment
20-1-12	25-4-12	28	50 grams of wheat in all cases.	A_1	9·7 10·2	35	97%
,,	16-5-12	,,		A_2	4·2 2·4	24	
,,	21-5-12	56		A_3	4·2 3·9	51	
,,	22-5-12	,,		A_4	19·2 19·5	45	
,,	23-5-12	85		A_5	21·3 20·6	40	
,,	27-5-12	,,		A_6	6·1 6·9	35	
,,	28-5-12	115		A_7	23·06 22·6	35	
,,	29-5-12	,,		A_8	11·0 10·4	39	
,,	31-5-12	170		A_9	20·5 20·7	35	
,,	4-6-12	,,		A_{10}	5·7 5·9	40	
,,	5-6-12	240		A_{11}	22·2 21·9	31	
,,	6-6-12	,,		A_{12}	12·4 12·8	33	
,,	6-6-12	28		B_1	14·6 14·2	38	
,,	10-6-12	,,		B_2	15·9 15·0	43	
,,	11-6-12	56		B_3	11·8 11·6	80	
,,	12-6-12	,,		B_4	14·2 13·7	80	
,,	13-6-12	85		B_5	12·9 12·8	81	
,,	14-6-12	,,		B_6	15·2 14·8	79	
,,	15-6-12	115		B_7	10·9 10·9	40	
,,	,,	,,		B_8	14·2 14·2	38	
,,	17-6-12	170		B_9	18·4 18·6	55	
,,	18-6-12	,,		B_{10}	17·0 17·6	63	
,,	19-6-12	240		B_{11}	31·0 30·8	52	
,,	,,	,,		B_{12}	28·7 28·9	60	

(B) A similar experiment was made on a mixture of weevil-infected and clean whole grain, a 50% mixture by weight.

Since the weevil-eaten grain is, however, lighter than undamaged wheat, and since the germination test is estimated by the number of grains germinating out of one hundred grains tested, it was necessary to estimate the number equivalent in a 50% mixture by weight; 20 grams of damaged and 20 grams of undamaged wheat were therefore counted and were found to contain the following number of grains of wheat.

Number of grains of wheat in 20 grams weight.

Sound	Weevil infected
696	808

That is in 100 grams of a mixture of wheat containing 50 grams of sound wheat and 50 grams of weevilled wheat (50% of each) there will be by count 53% damaged grains.

TABLE XXIV gives the summarized results of this experiment. Several points are brought out in this table. In the first place the results of experiment (A) above are confirmed in showing a marked diminution in the germinating power of both sound wheat and weevil-damaged wheat after remaining in contact with carbon dioxide. In addition also no numerical relationship is established between the amount of gas and the decline in vitality except in the case of the weevilled grain where the vitality falls off as the percentage of carbon dioxide increases.

In this experiment four blank tests were made—two in which mixed wheat was placed in bottles the necks of which were closed with cotton wool (bottles C_1 and C_2) and two closed with glass stoppers but to none of these four was any carbon dioxide added.

Only the bottles C_1 and C_2 contained live weevils at the end of the experiment but all the tests showed an increase in the number of weevilled grains.

From this it appears that even in an atmosphere containing 50% of carbon dioxide the weevils had extended their ravages to fresh grain before succumbing to the effects of the gas.

Since writing the above note my attention has been directed to Kidd's[1] work on the controlling influence of carbon dioxide on the maturation, dormancy and germination of seeds.

[1] Kidd. *Proc. Royal Society*, 1914. B. 87, 408—421, 609—625.

TABLE

The effect of carbon dioxide on a mixture of sound and

1	2	3	4	5	6
Date of charging with CO_2	Date of analysis	No. of CO_2 bubbles with which the bottle was charged	Contents of the bottle	Number of the bottle	Description of the bottle
23-4-12.	20-6-12	*Nil*	53·7 % damaged grains (by count.)	C_1	Bottle not closed, simply plugged with cotton wool.
,,	,,	,,		,,	,,
,,	21-6-12	,,		C_2	Bottle closed. No CO_2 added.
,,	,,	,,		,,	,,
,,	22-6-12	32	20 grams good wheat (696 grains) together with 20 grams (cent per cent weight) weevil infected wheat (808 grains). (Total number of grains being about 1504).	C_3	Bottle stoppered after charging.
,,	,,	,,		,,	,,
,,	24-6-12	64		C_4	,,
	,,	,,		,,	,,
,,	,,	96		C_5	,,
,,	,,	,,		,,	,,
,,	25-6-12	160		C_6	,,
,,	,,	,,		,,	,,
,,	,,	192		C_7	,,
,,	,,	,,		,,	,,
,,	26-6-12	256		C_8	,,
,,	,,	,,		,,	,,
,,	,,	320		C_9	,,
,,	,,	,,		,,	,,
,,	27-6-12	960		C_{10}	,,
,,	,,	,,		,,	,,

NOTE.—No weevils were found living on the date of analysis in any bottle,

XXIV.

weevilled wheat and on the weevils contained in it.

7	8	9	10	11	12	13
CO_2% absorbed by caustic potash	Germination % of good grains on the date of analysis	Germination % of weevilled grains on the date of analysis	Number of weevilled grains on the date of analysis	Number of good grains on the date of analysis	Germination % of good grains	Germination % of weevilled grains. (The wheat was cent per cent weevilled.)
0·14	62	28	898	633	...	...
0·18			59·7%	42·1%	...	...
17·84	82	81	972	595	...	...
17·55			64·6%	39·6%	...	...
20·18	45	15	905	600		
20·12			60·2%	39·9%	...	...
23·00	75	17	900	676	98	24
23·05			59·8%	44·9%	...	...
25·53	55	5	991	661	...	...
25·27			65·9%	43·9%	...	...
29·94	39	*Nil.*	935	635	...	...
29·99			62·2%	42·9%	...	...
28·54	51	*Nil.*	945	665	...	...
28·30			62·8%	44·2%	...	...
35·75	71	1	938	709	...	...
35·72			62·4%	47·1%	...	...
30·64	89	2	990	475	...	...
30·62			65·8%	31·6%	...	...
50·97	85	3	804	596	...	...
50·58			63·5%	39·6%	...	...

except in the first bottle C_1 where 66 weevils were still living.

This author shows that the germination of seeds is retarded or inhibited by the high partial pressure of carbon dioxide. In seeds which failed to germinate on this account the germinating power was restored by removing the testa and in some cases by completely drying and rewetting. He attributes this loss of germinating power to the reduction in the permeability of the testa under the influence of carbon dioxide resulting in a reduced amount of oxygen reaching the embryo and a corresponding rise in the carbon dioxide pressure in the embryo tissues.

Experiments with Sulphur dioxide.

In compliance with the wishes of Government the effect of sulphur dioxide on grain was tested. In 1912, the proprietors of the Faridkot grain stores reported that sulphur dioxide had been successfully used there for fumigation purposes. Harcourt[1] had already studied the effect of hydrocyanic acid, carbon disulphide and sulphur dioxide on the baking properties of flour prepared from wheat subjected to the action of these gases.

Hydrocyanic acid did little damage to the bread-making properties of the flour. Carbon disulphide spoiled the flour for a time giving it a darker colour and Harcourt considered from his experiments that it would take upwards of five months exposure to air to restore the qualities of the flour after treatment with this substance.

Sulphur dioxide totally destroyed the flour for bread-making purposes. This we should expect from a knowledge of the properties of the gas. Sulphur dioxide is a powerful bleaching agent and owes this property to its affinity for oxygen, it being one of the most active reducing agents known to the chemist. It moreover forms an acid, sulphurous acid, when brought in contact with water, and in the presence of organic matter such as wheat flour and moisture, rapidly oxidises to sulphuric acid. Both of these acids will materially affect the nature of the carbohydrates present in flour changing them to sugars of an hygroscopic nature, and if much sulphuric acid be formed, a darkening in colour will succeed the first bleaching action of the sulphur dioxide. Our experiments did not aim at confirming or extending the enquiries of Harcourt on the effect of this gas on flour but were to prove the inapplicability of the gas owing to its injurious effects on the vitality of the grain—and the destruction of this for seed purposes.

[1] Harcourt. Effect of Fumigants on Flour. *North West Miller*, **83** (1910), No. II, pp. 661—662.

,, *Annual Report of Ontario Agricultural College*, **35** (1909), p. 66.

Experiment.

Three sets of experiments were done. In the first five all samples of wheat received the same amount of sulphur dioxide added to the air of the bottle—using the method described above in the case of carbon dioxide. The gaseous mixture was allowed to remain in contact with the grain for periods varying from 48 hours to 144 hours. In the second five samples all received different amounts of gas varying from 100 to 600 bubbles of the gas and allowed to act in all cases for a period of 24 hours. In the third set the method of the second was followed but much larger quantities of sulphur dioxide were added as will be seen from the analyses in Table XXV.

TABLE XXV.

The effect of sulphur dioxide on the germinating power of wheat. (50 grams in a bottle of 150 cc. capacity.)

Number of experiment	Gas (SO_2) added for x seconds at the rate of 10 bubbles per second	Time during which the grain was exposed to the action of the gas	GERMINATING VALUE OF THE GRAIN before treatment with SO_2	GERMINATING VALUE OF THE GRAIN after treatment with SO_2	Amount of Sulphur dioxide and sulphurous acid calculated as SO_2 present at end of experiment	REMARKS
1	20 seconds	48 hours	97%	30	1·40	Volumetric method of Analysis used. SO_2 by Volume.
2	Do	72 do.	Do.	27	1·05	
3	Do	96 do.	Do.	23	0·	
4	Do.	120 do.	Do.	24	0·75	
5	Do	144 do.	Do.	21	0·53	
6	10 do.	24 do.	Do.	54	·23	
7	20 do	Do.	Do.	49	0·53	
8	30 do.	Do.	Do.	45	0·91	
9	40 do.	Do.	Do.	38	..	
10	50 do.	Do.	Do.	26	1·05	
11	60 do.	Do.	Do.	23	2·30	
12	30 do.	Do.	Do.	97	Nil	Absorption method of analysis used. SO_2 by Volume.
13	60 do.	Do.	Do.	97	Nil	
14	90 do.	Do	Do	65	Nil	
15	120 do.	Do	Do.	36	1·30	
16	150 do.	Do	Do.	32	3·60	
17	180 do.	Do.	Do.	38	11·96	

Two methods of analysis were followed, to check the amount of sulphur dioxide (or sulphurous acid) present at the end of the experiment. In the first dealing with small quantities of the gas, the contents of the bottle were agitated with boiled and cooled water, filtered, and a portion of the filtrate titrated with standard solution of iodine (decinormal). In the second, dealing with stronger mixtures, the gas was extracted from the bottle over mercury—and the amount of sulphur dioxide determined by absorption with dilute iodine solution in a Hempel's apparatus.

When the sulphur dioxide is as low as 0·23% by volume, and the time of exposure of the grain to this mixture only 24 hours, the vitality of this grain had fallen over 40%, thus showing the impossibility of treating seed wheat with this gas.

This closes what we may term the preliminary chemical enquiries on the subject and before further progress could be made it was necessary to examine the nature and habits of the various insect pests affecting wheat and generally referred to as "Weevils."

For this the services of Mr. A. J. Grove, Supernumerary Entomologist, were requisitioned and the results of his studies have already been given in Chapter I of this memoir. On these results have been based the experiments of Chapter III chemical experiments second series, Chapter V physical experiments connected with the effect of humidity—dryness—and heat on the weevils and Chapter VI remedial measures and experiments connected with these.

SUMMARY OF THE RESULTS OF THE EXPERIMENTS DESCRIBED IN CHAPTER II.

Briefly summarized these results are as follows :—

(*a*) The moisture content of Punjab wheat is not the same as described by Fletcher for wheat examined at Pusa, but on the other hand is shown to fulfil the conditions of immunity against weevil attack laid down by Lefroy and Fletcher.

Damage does, however, occur to stored wheat in this province in spite of this and we have shown elsewhere (Chapters I and V) that the other insects responsible for this *R. dominica* and *A. undulatus* do not react in the same way towards conditions of moisture and dryness as does *C. oryzæ* the insect on which Lefroy's and Fletcher's opinion is based.

(*b*) Our experiments confirm Badcock's opinion that wheat stored in carbon dioxide loses its vitality.

(*c*) The injurious effects of carbon dioxide and sulphur dioxide on the vitality of the seed have been numerically defined and the impossibility of

using either of these gases as insecticides for wheat which is stored for seed purposes, shown.

(*d*) The amount of carbon dioxide expired by germinating wheat is shown to amount to as much as 35% of the total weight of the grain when this is supplied with sufficient oxygen to maintain the process of germination in full activity, and as low as 4% when the supply of oxygen is restricted.

(*e*) That wheat is not a hygroscopic substance like starch and does not take up moisture from a damp atmosphere such as exists during the monsoon period in the Punjab in July and August to a greater extent than one or two per cent., and that moisture so taken up probably lies on or in the outer shell of the grain since it is easily removed by drying at a temperature of 70°C.

(*f*) Sun-dried wheat at harvest time contains about 8% of moisture which can be driven off at 100°C. and from 3½ to 4½% of moisture which can be driven off at 70°C.

CHAPTER III.

CHEMICAL INVESTIGATIONS 2ND SERIES.

Effect of Carbon dioxide, Hydrogen and Nitrogen on weevils.

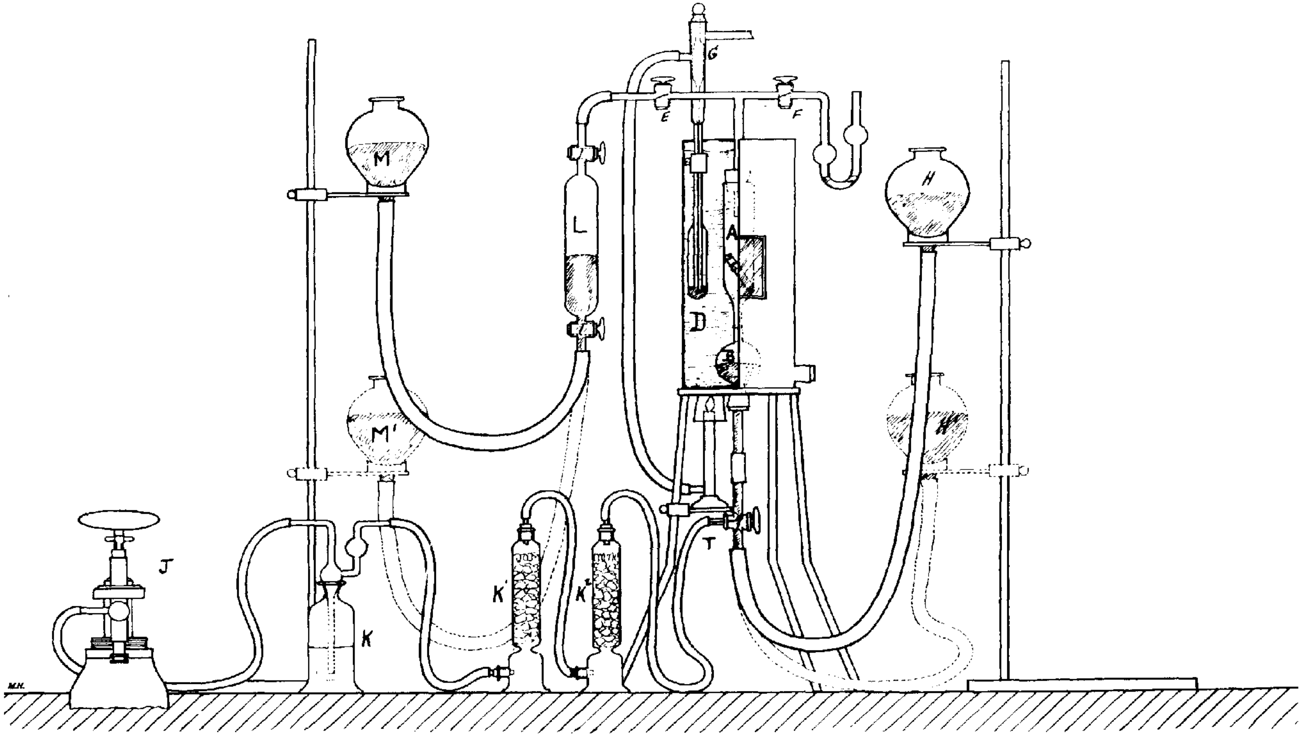
In February 1914, Mr. A. J. Grove was attached to the Punjab Agricultural staff to collaborate in the investigation and take up the entomological side of the enquiry in detail. Simultaneously with the study of the life-histories of the weevils detailed in Chapter I a more exact series of observations on the effect of gaseous insecticides was undertaken, confining ourselves in the first place to the gas carbon dioxide and working on the insects themselves. The three insects *Calandra oryzæ, Attagenus undulatus* and *Rhizopertha dominica* were the subjects of this series of experiments, as observation proved these to be the insects most active in causing damage in the Punjab.

No systematic study of the process of respiration of insects from a chemical and physiological standpoint seems to have been undertaken by previous workers. Graham[1] concludes that respiration in insects follows the law of gaseous diffusion, but this seems based only on the fact that the aeration of tissues in insects is effected by means of a series of fine air tubes, trachæ, spreading to all parts of the body and having their openings in the form of spiracles on the sides of the body, and that these small openings and fine tubes offer the equivalent of the porous diaphragm of Graham's experiments in gaseous diffusion. Anatomical data as well as observations on the living animal, however, show that the opening and closing of these spiracles as well as the volume movement of the air tube is under muscular control, and biologists have been satisfied with this in the absence of further physical support of Graham's view, namely, that an exchange between the gases of the tube and the outer air is effected by means of muscular pressure.

Knowledge of the process seems to stop short at this reasonable explanation of gaseous exchange and no attempt has been made to examine the processes by which the insect makes use of the air thus brought in contact with its body tissues. The intake of air and the exhalation of carbon dioxide has been not unnaturally assumed, and the detailed study of the chemical processes

[1] *Researches*, p. 44.

PLATE V.



Apparatus used in examining the effect of carbon dioxide, hydrogen and nitrogen on weevils.

of respiration left to the physiologist. This science has until very recent times been of interest only to students of medicine, its study has consequently been confined largely to the higher animals. These with their increased morphological differentiation of structure render the physiological problems more complex and more obscure and it is often in the study of simpler organisms that light is thrown on some process common to all. This has proved to be so in the present case. We shall deal with the experiments in the order in which they were made.

The first step taken was to examine Cole's results in detail, namely the effect of carbon dioxide on the three insects enumerated above, testing the effect of the gas at different temperatures and extending the experiments to other gases of a probable inert nature like nitrogen and hydrogen in order to test Graham's theory.

The apparatus used is shown in detail in Plate V. It consists of a glass incubating chamber A in which the insects are contained in small cages (C). These cages are only glass tubes closed at either end by wire gauze caps, and in them, the number (usually 10) of insects experimented upon are enclosed. The incubating chamber A is connected with a glass bulb B by means of a capillary tube. The object of this bulb is to provide space in the incubating chamber for the admission of mercury, to drive out some of the gases of the chamber for the purpose of analysis, without running any danger of interfering with the composition of these during the experiment. The volume of B is about 75 cc. while the volume of the tube A is about 150 cc. A and B are closed above by a rubber bung and below by a three-way tap T and at the upper end are in connection with a T-shaped capillary tube—one end of which has a small mercury manometer, which, by means of tap F allows the gas pressure in A and B to be reduced to atmospheric level, and the other side through which the gases can be withdrawn for analysis into the gas tube L for transfer to the gas analysis apparatus. The whole apparatus is in a constant temperature bath D, the temperature of which is regulated by the thermo-regulator G.

Some difficulty was experienced in the use of mercury thread regulators of the type shown at G on account of the College gas supply being oil gas at high pressure (Mansfield's system) the working pressure of which is equivalent to 6″ of water pressure on the gas holder. This was satisfactorily overcome by placing a 10-litre bottle containing some 6 inches of water in it, between the gas tap and G, and passing the gas through the water by a tube, the lower opening of which was 4″ under water; this gave a gas pressure in the bottle

of $(6''-4'')=2''$ a satisfactory pressure to work the thermo-regulator and the small flames required for maintaining the bath at constant temperature.

In charging the apparatus the tube A B is first cleaned, dried and placed in position in the empty water bath. After the introduction of the cages C containing the insects, the T tube EF is attached to the tube A B with a rubber bung. The gas (carbon dioxide, etc.), the effect of which we wish to test, issues under its own pressure from a steel cylinder at J, and, after passing through the purifying vessels K, K_1, K_2 enters the incubation chamber A B through the three-way tap T—the mercury reservoir H having first been lowered to the position H_1 to allow this. Gas is passed through for 15 to 20 minutes to entirely displace the air upwards (when filling the tube with hydrogen the gas enters at E and displaces the air downward through T which is then left open). After sufficient time has elapsed to displace the air, E is closed, and F opened, and a few bubbles of the gas passed through the manometer to clear that tube of air. B is then put in communication with H by turning T and the tube immediately above T filled with mercury. T is then closed and H_1 raised to H.

Lastly F is closed, and the water bath D filled with water at the temperature of the experiment. In order to check any change in the composition of the gases in A and B during the experiment, changes induced by the insects themselves if large enough to be detected by the analytical means at our disposal, or changes resulting from accidental leakage, the gas in A B is analysed after the last operation noted above, *viz.*:—the closing of the tap F—the time of the commencement of the gas treatment. The method of extracting and analysing the gases is simple. A gas tube L closed at either end with taps, and extending into capillary-bore tubes is attached to E by thick walled rubber tubing on the one side, and to a mercury vessel M on the other. L is first filled with mercury, and M is then lowered to M_1, by slowly opening T mercury from H flows into B putting the gases of A B under pressure, and on opening E these flow into L—the first gas flowing into L from the tube outside E is rejected. After about 50 cc. of gas have been transferred to L, T is closed, leaving the gases in A B under a little more than atmospheric pressure, which latter is finally reduced to atmospheric pressure by opening F. Before doing so however the capillary tube outside E is filled with mercury by first closing the upper tap of L and forcing mercury in to put the gas in L under pressure. The lower tap of L is then closed and the tube inverted. On opening the upper tap, mercury from L now flows into E—all taps are now closed and L is disconnected and removed to the gas analysis apparatus and the sample introduced and analysed in the usual manner.

The apparatus used in these experiments for the analysis of the gases was first Bone's modification of Frankland's apparatus, but experience showed the accuracy of this method of working was really beyond that required. In view of the tediousness of the method we subsequently substituted Macfarlane and Cadwell's modification of Sodeau's apparatus.[1]

After the lapse of some hours the gases of the tube A B were again analysed and the apparatus opened to examine the insects and determine the number which had been killed. The first effect of carbon dioxide was to induce intense activity in the insects for the period of a few seconds, after which they became inert and without motion. It was found however that this was not death, for on restoring them to an air atmosphere they recovered. We determined the *time*, *temperature* and *gas concentration* necessary to produce death and the time so taken to kill the insects we have called the *lethal period*. To determine this lethal period it was necessary to make many experiments before the exact period could be arrived at, and in the case of carbon dioxide at a temperature of 35°C, upwards of 60 experiments on each insect and 120 analyses of the tube gases were required before the lethal period was determined. As some of the test periods were upwards of 60 hours it will be seen how lengthy and tedious was the method of investigation, for in the table which follows only one figure appears for all these analyses and tests.

The following table XXVI shows the method of recording the result of one test on the three insects.

Duplicate tubes of each insect were taken—each tube containing 10 insects and the number given in column 5 shows the number *recovering*. The difference between this number and 10 will be the number of insects killed.

TABLE XXVI.

Example of the method of investigating the effect of carbon dioxide on the insects **Attagenus undulatus** *and* **Calandra oryzæ.**

Date	Analyses of the gas before and after CO_2 %		Number of hours of treatment	Number of insects recovering		Types
	Before	After				
1-9-14	99·8	99·78	11 hours	9	1	*A. undulatus.*
	99·92	99·89	10½ ,,	7	3	
	99·96	99·93	10 ,,	2	2	
21-11-14	99·5	99·5	2½ ,,	0	0	*C. oryzæ.*
	98·4	98·4	3 ,,	0	1	
	98·3	98·3	4 ,,	0	0	

[1] J. C. S. I. Feb. 28, 1903, p. 187.

TABLE XXVII.

Showing the **Lethal period** *for Attagenus undulatus, Calandra oryzæ and Rhizopertha dominica* **in an atmosphere of dry carbon dioxide.**

TEMPERATURE	ATTAGENUS UNDULATUS.				CALANDRA ORYZÆ.				RHIZOPERTHA DOMINICA.			
	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)
	Before	After			Before	After			Before	After		
30°C ...	99·00	98·90	89	1·3720	98·8	98·8	30	1·3718	98·60	99·00	14	1·3720
30°C	99·6	99·60	63½	1·3786	99·7	100	51½	1·3770	99·80	99·70	50	1·3758
35°C	98·0	98·20	37	1·3459	98·9	98·2	17	1·3459	99·56	99·19	22	1·3529
40°C ..	99·56	99·61	12½	1·3324	98·1	98·1	3½	1·3256	99·70	99·42	7	1·3326

TABLE XXVIII.

Showing the **Lethal period** *for Attagenus undulatus, Calandra oryzæ and Rhizopertha dominica* **in dry hydrogen.**

TEMPERATURE	ATTAGENUS UNDULATUS				CALANDRA ORYZÆ				RHIZOPERTHA DOMINICA			
	Analysis of Gas		Lethal period in hours	Density of gas (air at 0°C=1)	Analysis of Gas		Lethal period in hours	Density of gas (air at 0°C=1)	Analysis of Gas		Lethal period in hours	Density of gas (air at 0°C=1)
	Before	After			Before	After			Before	After		
30°C ...	95·8	95·9	28	·09599	96·3	96·10	27	·09275	95·75	94·7	16	·0973
35°C ...	96·5	96·4	22	·08961	90·3	90·09	11	·13925	90·10	89·8	14	·14083
40°C ...	95·3	96·1	16	·09133	96·1	96·10	2	·09133	96·10	96·1	4	·09133

TABLE XXIX.

Showing the **Lethal period** *for Attagenus undulatus, Calandra oryzæ and Rhizopertha dominica* **in dry nitrogen.**

TEMPERATURE	ATTAGENUS UNDULATUS.				CALANDRA ORYZÆ.				RHIZOPERTHA DOMINICA.			
	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)	ANALYSIS OF GAS		Lethal period in hours	Density of gas (air at O°C=1)
	At beginning	At end			At beginning	At end			At beginning	At end		
30°C	100	100	55	·8764	99·9	99·8	28	·8764	99·6	99·7	31	·8764
30°C	98·8	100·1	20½	·8764	100·8	99·5	17	·8764	99·7	99·4	16	·8764
35°C	99·6	99·0	30	·8620	99·7	99·6	11	·8620	99·6	99·7	16	·8620
40°C	99·9	100	13	·8484	100	100	4	·8484	99·0	99·7	6	·8484

In all these experiments dry gases were used. The time at our disposal did not allow of our investigating the lethal period for the same gases in a moist condition. We investigated the effect of dry and moist air however and this will be described in Chapter V. The results are shown in tabular form for the three gases carbon dioxide, hydrogen and nitrogen in Tables XXVII, XXVIII and XXIX.

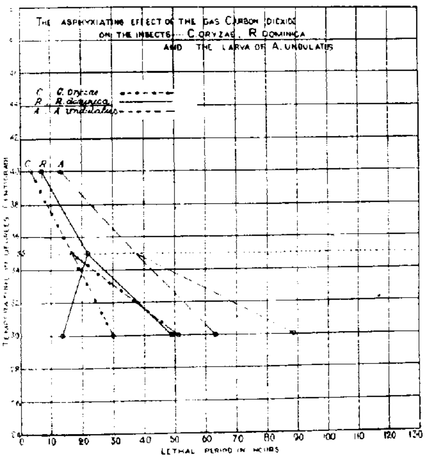


Fig. 4

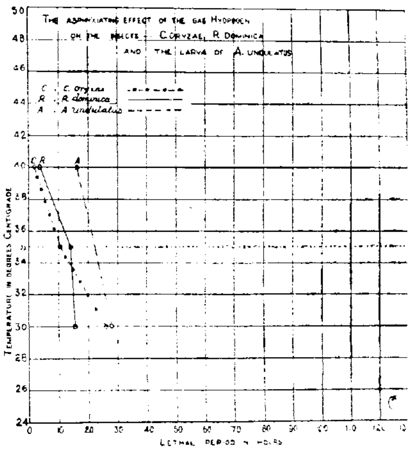


Fig 5

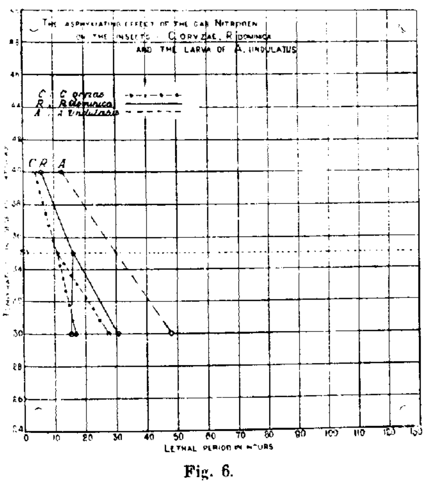


Fig. 6.

Several points are brought out in thes tables. The first is the wide discrepanc between the recorded duplicates which wer done *at a different season* of the year. Thi shows that the effect of these gases on th insects differs very considerably at differen times of the year—it is not *temperature* her that matters, or the composition of the ga —it is the *condition of the insect*; we sha refer to the point in more detail later. Th second point brought out in the results is th general shortening of the lethal period as th temperature rises—this is most marke in the case of *A. undulatus* with carbo dioxide.

Thirdly, *A. undulatus* seems to be more resistant to all these gases than *R. dominica* and *R. dominica* more resistant than *C. oryzæ*.

Fourthly, the lethal period is much shorter in all cases as the density of the gas applied decreases. On first sight this latter seems to give support to Graham's theory that the respiration of these insects follows the law of gaseous diffusion.

The figures were therefore closely examined as follows:—

The following table gives the density of these gases at 0°, 30°, 35° and 40°C. as compared with air at 0°C:—

TABLE XXX.

Density of air, hydrogen, nitrogen and carbon dioxide compared with air, density=1 at 0°C.

Gas	T=0°C	T=30°C	T=35°C	T=40°C
Air	1·0	0·90120	0·8863	0·87240
Carbon dioxide ..	1·52900	1·37700	1·3550	1·33400
Hydrogen	0·06949	0·06263	0·0616	0·06063
Nitrogen	0·97240	0·87610	0·8620	0·84840

From these figures and a knowledge of the composition of the gas in the tube, we can calculate the density of the gas actually present in the tube during the experiment. For example in the case of *Attagenus undulatus* at 30°C. the analysis of the gas was hydrogen 95·9—nitrogen 4·1.

The density of this mixture would therefore be

$$\frac{(95{\cdot}9 \times {\cdot}06263) + (4{\cdot}1 \times {\cdot}8764)}{100} = 0{\cdot}09599$$

If the lethal period is related to the rate of diffusion then according to Graham's law the following relation should hold:—

$$\frac{\text{Lethal period in carbon dioxide}}{\text{Lethal period in hydrogen}} \propto \sqrt{\frac{\text{Density of hydrogen}}{\text{Density of carbon dioxide}}}$$

$$\text{Since } \frac{\text{Velocity of diffusion of carbon dioxide}}{\text{Velocity of diffusion of hydrogen}} = \sqrt{\frac{\text{Density of hydrogen}}{\text{Density of carbon dioxide}}}$$

Example.—Comparison of the lethal periods for *Attagenus undulatus* at 30°C. with the diffusion ratios of the gases in which the lethal period was determined, and calculated from the densities of the gases.

By analysis	Density of carbon dioxide at 30°	..	1·3459
	Density of hydrogen at 30°	..	0·0896
	Density of nitrogen at 30°	..	0·8620

$$\frac{\text{Velocity of diffusion of carbon dioxide}}{\text{Velocity of diffusion of hydrogen}} = \sqrt{\frac{0{\cdot}0896}{1{\cdot}3459}} = 0{\cdot}2583$$

$$\frac{\text{Velocity of diffusion of carbon dioxide}}{\text{Velocity of diffusion of nitrogen}} = \sqrt{\frac{0{\cdot}862}{1{\cdot}3459}} = 0{\cdot}80113$$

$$\frac{\text{Velocity of diffusion of hydrogen}}{\text{Velocity of diffusion of nitrogen}} = \sqrt{\frac{0{\cdot}861}{{\cdot}0896}} = 0{\cdot}3102$$

The reciprocals of these velocity diffusion ratios are :—

0·2583	reciprocal	3·87
0·80113	reciprocal	1·24
0·3102	reciprocal	3·22

The lethal periods in the same experiment were as follows :—

Carbon dioxide.	Hydrogen.	Nitrogen.
37 hours.	22 hours.	30 hours.

$$\text{Ratio } \frac{CO_2}{H} = \frac{37}{22} = 1{\cdot}7$$

$$\frac{CO_2}{N} = \frac{37}{30} = 1{\cdot}23$$

$$\frac{H}{N} = \frac{22}{30} = 0{\cdot}73$$

After a similar examination of all the results of Tables XXVII, XXVIII and XXIX we are unable to obtain any support of Graham's theory that the respiration of insects is regulated by the laws of gaseous diffusion.

The velocity ratios calculated from the gas analysis figures show that temperature will have but little effect on this *comparative* value, since for the small increase in temperature covered by the range of our experiments the small difference in the mass of the reacting gas molecules will have but a slight effect on the kinetic energy of the molecule, both gases being heated through the same range of temperature.

Table XXXI.

Diffusion velocity ratios calculated from the composition of the gases used to test the lethal period for Attagenus undulatus.

	Velocity of CO_2 / Velocity H.	Velocity of CO_2 / Velocity N.	Velocity of N. / Velocity H.
30°C	0·2641	0·798	0·302
35°C	0·2583	0·80113	0·3102
40°C	0·2628	0·798	0·305

If then the composition of the gases used in these experiments is so constant that the diffusion ratios remain as close as is shown in the table, the lethal period ought to show a like similarity *if the exchange of gases through the spiracles and air tubes of the insect follows the law of gaseous diffusion.* Even if there are two or more factors making up the lethal effect—and it is difficult to conceive such inert gases as nitrogen, hydrogen and carbon dioxide acting otherwise than as asphyxiating agents, this diffusion factor will at least remain constant.

The only deduction we are able to draw however is that the lethal effect increases with the temperature and that the effect therefore is in all probability of the nature of a chemical reaction. This view seems to receive support in the wide variation observed in the case of the gas carbon dioxide which in the first series of experiments made at 30°C. gave a lethal period of 89 hours. This latter result was obtained in an experiment conducted in the month of March, while the period of 65 hours was determined in the following autumn.

CHAPTER IV.

Respiration.

Historical. The experiments detailed in Chapter III have yielded results so incomprehensible, and so incapable of interpretation, that further enquiry was made into the possibility of these gases entering into, or interfering with, some cycle of chemical changes—normally taking place in the process of animal oxidation or respiration. It seems very unlikely that the gases themselves have taken part in any of the reactions connected with this process, since no alteration in the composition of the gas was detectable in the course of the experiment. On the other hand it must be conceded that the amount of gas brought into play by the number of insects experimented on, is probably so small compared with the total amount of gas present in the incubation tube, that the analytical methods at our disposal were too crude to detect the minute change, if any, which had taken place.

In order to find a satisfactory explanation we therefore turned to an examination of the process of respiration and more especially respiration in the cell as a unit of the entire organism. Historically Lavoisier was the first to recognize the importance of oxygen in the life process, and he outlined the part played by this substance in the combustion processes taking place in animals. Lavoisier believed the lungs to be the seat of the oxidation processes taking place in the animal organism. This was not feasible because the energy so set free would not be available for the tissues of the body, and the cells comprising them. Magnus,[1] by analyses of the blood, and the gases of the blood, showed this to contain oxygen until the final capillaries were reached when it disappeared. He thus proved that all animal oxidation did not take place in the lungs but his researches left undecided whether the oxidation took place in the blood itself, or whether the oxygen passed through the wall of the blood vessels into the tissues. Ludwig and Schmidt[2] imagined that the tissues were constantly giving up oxidisable substances to the blood, and in support of this showed that the restriction of an animal's supply of oxygen led to

[1] Magnus. *Ann. Physik,* **40**, 583. 1837.
" " " **64**, 177. 1845.

[2] Ludwig and Schmidt. *Ber. über die Verhandl. der Sächs. Ges. Wissen. Leipzig. Math Physikal Klasse,* **19**, 99. 1867.

suffocation; that the blood of such suffocated animals contained but traces of oxygen, and that on exposing the blood to the air or oxygen, the latter disappears, and the amount of carbon dioxide in the blood increases. They considered that in a suffocated animal these oxidisable substances given up to the blood by the tissues, accumulated.

We now know that blood contains cells, the red and white corpuscles, which themselves undergo metabolism, and thereby very easily consume oxygen and give out carbon dioxide. Afonassiew[1] then showed that only the blood corpuscles, and not the serum of a suffocated animal, could thus take up oxygen. The assumption that the combustion takes place in the tissues and cells of the tissues, was proved by Pflüger and Oertmann[2] in the following manner. A frog's blood was removed and replaced with normal saline solution. The animal was then placed in an atmosphere of pure oxygen, and consumed as much of the gas and evolved as much carbon dioxide as a normal frog.

To-day there is no doubt that oxygen diffuses into the tissues, and that the cells themselves derive their energy by the combustion of nutrient substances in them. One of the principal proofs of this is, that the blood itself possesses no oxidising properties.[3] For example, salts of lactic acid placed in the blood remain unchanged whereas in their passage through the organism they are completely and rapidly oxidised. This is the more convincing when carried out on surviving organs. If for example blood is conducted through the liver of a dead animal by the portal vein, it can be shown that ammonium formate introduced into the blood, disappears, and in its place urea is formed.[4] This is not the case if the ammonium formate is brought only in contact with the blood. Contact with the liver cells is essential.

That oxygen actually passes through the walls of the blood vessels is strikingly shown by the way the fœtus is provided with this element. It is well known that there is no direct connection between the vascular system of the mother and of the child. The circulation of the fœtus is isolated.[5] Bancroft[6]

[1] Afonassiew. *Ber. über die Verhandl. der Sächs. Ges. Wissen. Leipzig. Math-Physikal Klasse*, **24**, 253. 1872.

[2] Pflüger and Oertmann. *Pflüger's Arch.* **15**, 382. 1877.
„ „ „ „ „ **10**, 251. 1875.

[3] Pflüger. *Pflüger's. Arch.* **6**, 43. 1872.
Hoppe-Seyler. „ „ **7**, 407. 1873.

[4] Abderhalden. *Text-book of Physiological Chemistry*, translated by Hall. Wiley and Sons, New York (1908).

[5] Pflüger. *Pflüger's Arch.* **1**, 686. 1868.
Kulz. *Zeit. Biolog.* **23**, 321. 1887.

[6] Bancroft. *Biochem. Jour.* I. I., 1906.

has shown that saliva contains 0·5% of oxygen by volume, which could only have come from the circulation by diffusion.

All this gains further support when we come to examine the system by which the lower organisms receive their oxygen supply in a direct form. In insects there is a modified vascular system and the oxygen reaches the tissues in the gaseous form through an infinitely branched tracheal system.

While all these observations tend to show that the higher organisms are capable of taking up and directly using the oxygen of the air, quite a separate series tend to show that they can obtain energy from certain hydrolytic cleavage processes. It is known that intestinal parasites live apart from an oxygen containing atmosphere and even frogs can for a time[1] live without oxygen and produce carbon dioxide. Though this is possible we know that the energy derived from such cleavage processes is insufficient for the production of energy required for the animal processes. Fick and Wislicenus[2] proved this in an interesting series of experiments on themselves in mountain climbing. If the animal cell is capable therefore of existing on the energy derived from cleavage processes, as well as utilizing atmospheric oxygen for the oxidation processes when an additional expenditure of energy demands the more rapid combustion of the cell nutrients, it follows that the cell is a facultative anaerobe. Here we find a parallel in the unicellular organisms, where there exist not only bacteria which demand the presence of oxygen, or can exist in its absence (aerobic and facultative anaerobic bacteria), but others, the anaerobic bacteria to which oxygen acts either as a deterrent or even as a poison. It is a characteristic function of all bacteria that they evolve carbon dioxide whether they take up oxygen from the air or not. Exception.—The acid forming bacteria—the vinegar bacteria—may evolve no carbon dioxide when oxidising an abundance of alcohol to acetic acid. The bacteria which are anaerobic or temporarily so, must derive their oxygen from the nutrient material on which they subsist or to put it more exactly they derive the energy necessary for the vital processes from the partial decomposition of chemical substances rich in oxygen. Examples, decomposition of grape sugar by the *Saccharomycetes*, the reduction of nitrates in the presence of organic matter by anaerobic bacteria.

We are not further concerned here with the exact function of the blood corpuscles, or the serum in which they exist. It is sufficient for our purposes in

1 Pflüger and Oertmann. *Pflüger's Arch.* **15**, 382. 1877.
" " " " " **10**, 251. 1875.

2 Fick and Wislicenus. *Vierteljahresschrift des Züricher naturforschenden Gesellsch.*, **10**, 317. 1865.

this research to note in passing that in the higher animals, these serve the purpose of conserving an oxygen supply through its absorption by hæmoglobin, and that the gas then passes *via* the blood plasma, and diffusion into the tissues of the body and the cells comprising them.

So far then we establish a parallel between the cells of all organized structures, either animal or vegetable, namely, that the oxygen of respiration reacts in the tissues in solution dissolved in the cell plasma. Whether it reaches the individual cell in the gaseous form as in the insects with their highly branched tracheal system, or through the medium of a loose chemical combination such as oxyhæmoglobin and from this *via* the blood plasma does not concern us here. We have now to examine the conditions under which the oxygen in solution in the cell can perform this process of oxidation, which as we have seen above in the oxidation of ammonium formate can be effected by the liver cells but not by the blood, though this latter is infinitely richer in oxygen than the former. (For a full historical account of this process of blood aeration *see* Abderhalden's *Physiological Chemistry*, 1908, London, Chapman and Hall, Chapter XVIII.)

If we expose such substances as fats, albumins, and carbohydrates, which normally constitute the "nutrient" material of the cell, to the action of oxygen at body temperature, no perceptible oxidation of these materials takes place. On the other hand within the body itself they are rapidly oxidised with the production of carbon dioxide, urea, and water. Consequently conditions must prevail within the cell which facilitate the action of oxygen upon the material exposed to its action.

We are acquainted with a number of facts which prove that even within the animal tissues oxygen as such is unable to act upon unchanged food. We know that in the process of respiration it is the food fuel which is consumed and not the cell substance. Under certain conditions (disease) the body may lose the power to deal with certain food susbtances such as carbohydrates. In diabetes, substances hard to oxidise are as easily consumed as when the body is in a healthy condition. Only unchanged dextro-glucose has ceased to act as a food, because the organism has lost the power to utilize it.

If grape sugar is but slightly changed before its introduction, the diabetic tissue is then able to deal with it.

If animal oxidation took place merely as a result of the coming together of oxygen and nutrient, then an increased supply of oxygen should be followed by a more vigorous oxidation, but this is not the case. Under normal conditions it is not possible to increase the amount of oxidation taking place in the tissues by increasing the amount of oxygen given to the animal.

The fact that oxygen in the condition in which it exists in the tissues is incapable of consuming the unchanged nutrient, enables the cell to adjust its metabolism to its requirements, above all it enables the cell to utilize certain and particular materials to suit its requirements.

Up to this point, no clear explanation as to the nature of animal oxidation processes has been put forward. We have recognized only the initial materials food and oxygen, and the final products of the combustion, carbon dioxide, water and certain nitrogen-containing substances such as urea.

The following hypotheses have been formulated. It has been suggested that the oxygen has been changed in form so that it more readily attacks the nutrient. Schönbein[1] attributes the numerous oxidations taking place in the plant organism to the primary formation of this changed oxygen from ozonizing material present in the plant. This assumption breaks down for want of experimental proof of the presence of ozone in the plant cell, and from the fact that small amounts of this substance are poisonous to the cell protoplasm. Hoppe-Seyler[2] assumed the presence of active oxygen in tissue and based his assumption on the fact that in animal tissues energetic reduction takes place side by side with oxidation. In this way reducing substances are formed which unite with one atom of the oxygen molecule, setting the other atom free. The butyric fermentation of glucose is an example of such a reduction.

$$C_6H_{12}O_6 = C_3H_7COOH + 2CO_2 + 2H_2$$

The assumption receives support in the theory of nitrification in which we assume that the organism responsible for nitrification first produces readily oxidisable substances which then decompose the atmospheric oxygen molecule and thus form nascent (active) oxygen for the oxidation of nitrogen.

If we are to believe that oxidation in the animal substance takes place in this way we must assume first of all that the food substances are hydrolyzed, and that easily oxidisable substances are then formed which are oxidised by the oxygen received in the tissues from the blood and *at the same time part of the oxygen is rendered nascent.*

The theory breaks down for want of experimental proof. We could understand why the diabetes cannot oxidize (*d*) glucose by assuming the absence of the necessary hydrolyzing ferment for the sugar, but we cannot understand why the nascent oxygen produced from the oxidation of the other food substances is unable to act on the sugar.

1 Schönbein. *Poggendorf's Annalen.* **65**, 171. 1845.
2 Hoppe-Seyler. *Pflüger's Arch.* **12**, 16. 1876.

There are certainly present in the cell a number of ferments which are specific—that is to say, induce chemical reaction according to the class of compounds on which they react, and if we assume that the cells do not contain these ferments in an active condition but become active under such conditions as the cell economy may demand, we can understand how the cell may contain food, ferment and oxygen without any oxidation taking place. The cell consumes the cleavage products of the food it obtains, such as the decomposition products of albumin, glycocol alanin, etc.,—oxidising these to urea. But this is only true of substances having the same molecular structure and configuration—thus a rabbit fed with *d.l.* leucine oxidises only the *l.* leucine, the other half of the racemic molecule *d.* leucine appearing unchanged in the urine. This is because the cells contain no ferment capable of dealing with *d.* leucine and so it remains unoxidised. (*cf.* Abderhalden's *Text-Book, Physiological Chemistry*, p. 443).

In the absence of sufficient oxygen we should expect to get an accumulation of fermented and readily oxidisable substances in the cell, particularly as the entire energy of the animal depends on that set free in hydrolytic decomposition.

Bunge[1] has shown that ascarids can exist several days without any oxygen supply, and we have already quoted Pflüger's experiments with frogs. In such cases as these we should expect to find some evidence of such easily oxidised substance as hydrogen if the animal energy is derived from partial decomposition of the food substances. In the case of ascarids this was not found by Bunge to be the case. No hydrogen could be detected, nor did oxygen disappear if supplied to the worms after they had existed for a day without it. Many cases are known in which the animal organism is protected from poisons by their oxidation in the cell, and it is known, too, that there may exist in the cell easily oxidisable substances. The assumption of the theory of active oxygen therefore cannot be said to have got beyond the hypothetical stage (Abderhalden). We must therefore look for a hypothesis which includes the known facts particularly the fact that the cell seems capable of exercising the function of *selective* oxidation.

Such an hypothesis must rest upon the presence in the cell, of ferments which regulate the preparation of food for oxidation, and secondly its oxidation by the oxygen in solution in the cell juices.

[1] Bunge. *Zeit. Physiology. Chem.* **14**, 318. 1890.

This was first suggested by Traube[1] who assumed the presence of an oxygen carrier. Schmiedeberg[2] has mentioned the possibility of such ferments and Jacquet[3] has proved that extracts of organs can act as carriers of oxygen, and that the *principles* causing this can be precipitated by alcohol and destroyed by heating to 100°C.

During the last 15 years a large number of such ferments have been detected in both animal and vegetable tissues, capable of assisting in the oxidation of a number of organic substances of a more or less stable nature. Palladin has examined the pigments produced by the respiration of plants and summarized the processes as follows :—

Primary process.	*Secondary process.*
Anaerobic enzyme zymase.	Oxygen
Katalase, Reductase.	Respiratory oxidase, phytohæmalin.
Fermentation products alcohols and other bodies.	Respiratory products.
	Carbon dioxide and hydrogen.

Bach[4] summarizes the recent knowledge on the subject of oxidising enzymes in cell oxidation.

(1) In order to utilize the oxygen of the air to effect oxidation an enzyme (an *oxygenase*), is secreted, which is readily oxidised fixing molecular oxygen to form a peroxide.

(2) A second enzyme (the *peroxydase*) which accelerates the oxidising action of the peroxides by acting on them in a similar way to ferrous sulphate on hydrogen peroxide.

(3) The peroxides are readily transformed by hydrolysis into hydrogen peroxide which is also formed as a primary product during hydrolytic oxidation. Owing to its rapid rate of diffusion the accumulation of hydrogen peroxide might damage cell protoplasm, to guard against this the cell produces an enzyme *catalase* which rapidly decomposes hydrogen peroxide into water and inert oxygen. *Catalase* thus acts as a regulator of the respiratory process.

(4) To effect hydrolytic oxidation an enzyme *perhydridase* is present, which accelerates both oxidation and reduction just as do the metals of the platinum group.

1 Traube. *Theorie der Ferment Wirkungen*, Berlin, 1858.
2 Schmiedeberg. *Arch. Exper. Path. Pharm.* **14**, 288. 1881.
3 Jacquet. „ „ **29**, 386. 1892.
4 Bach. *Arch. Sci. Phys. Nat.* **35**, 240—262. 1913.

The *reductase* consists of the enzyme, water, and air oxidisable substances, which fix the oxygen derived from the water, leaving the hydrogen free to effect reduction[1].

Bach and Chandal's theory with regard to the mechanism of cell oxidation and more particularly oxidation in the vegetable cell has been examined and criticised by Moore and Whitley[2].

These authors as a result of their investigations show a difference between the oxidising action of various vegetable juices and conclude that all such juices show the presence of only one type of enzyme engaged in the process of oxidation, this they term a *peroxidase*. They moreover suggest that this enzyme synchronises with the class of hydrolytic ferments and with the active bodies in natural and immune sera in forming a connecting link between them.

In all three classes of enzymic action three interacting bodies are required. In the case of the hydrolysing enzymes there is (*a*) the *combining substrate*, the food stuffs, carbohydrate, or fat (*b*) the *combining body*, the elements of water, finally—intermediary acid or alkali, in the presence of which alone the ferment is active (*c*) the *catalyst*, one of the digestive or other hydrolytic ferments, example, *pepsin*, *trypsin*, *amylase*, *zymase*, *lipase*.

In the case of *oxidising ferments* we have (*a*) *substrate*, the oxidisable substances, such as tyrosin, the naturally occurring phenols in the plants, or the chromogenetic indicators used by Moore and Whitley in their experiments; (*b*) *the combining body*—oxygen yielded either by hydrogen peroxide or by organic peroxides; (*c*) *the catalyst*, enzymes such as *tyrosinase*, *laccase*, etc.

In the case of immune sera there is (*a*) the *substrate*, the cell or bacterium to be dissolved or the toxic or foreign substances to be attacked or rendered inert, (*b*) the *combining body*—the complement or thermo-labile substance in the absence of which the reaction cannot proceed, and (*c*) the *catalyst*, the specific immune body or anti-body which attacks and disintegrates the foreign cell, or neutralizes the toxic substances.

One of the most interesting and important of these ferments from the point of view of this paper is the so-called *tyrosinase*. This substance was first found by Bertrand[3] in many genera of the Fungi in which it is associated with another oxidase *laccase* which however only acts on quinol and pyrogallol.

[1] *Abstract Jour. Chem. Soc.* CIV, 1913, p. 543.

[2] Moore and Whitley. Properties and Classification of the Oxidising Enzymes. *Bio Chem. Jour.* 1909, p. 136.

[3] Bourquelot and Bertrand. *Jour. Pharm. Chem.* **6**, 3, 177. 1896.
Bull. Soc. Mycol. France, **18**, 27, 1896.
Compl. rend. **122**, 1132, 1215. 1895.

Tyrosinase acts on tyrosin (hydroxyphenyl alanin) in the presence of oxygen or air forming a dark blue black solution (melanine). The dark colour of old and broken fungi is due to this oxidised tyrosin. The same or else a similar enzyme has been identified in the stomach juices of starved meal worms by Biedermann[1] which will act upon tyrosin. The darkening in colour of some insects after death, the so-called melanosis, has been shown by Von Furth and Schneider[2] to be the result of the action of a similar oxidase ferment. The larva of *Attagenus undulatus*, one of the insects infecting wheat in the Punjab, shows marked evidence of melanosis after death :—while living the larvæ are pale yellow or almost white in colour with a tendency to red. After death this changes to a dark brown. The presence in both vegetable and animal tissues of oxidase ferments capable of bringing about the oxidation of such inert substances as tyrosin which are able to resist all but the most powerful of the reagents at the disposal of the chemist, indicates that they play an important part in the process of cell oxidation—the final stage of respiration, but further evidence seems wanting to show that respiration is actually due to the presence of these ferments, that it is in fact an enzymic process.

To prove this successfully we must produce the ordinary effects of respiration under conditions in which the animal as a living organism has been destroyed, but in which any ferments present in the tissues have been left unimpaired, and secondly, we must show that the destruction of the enzyme results in the checking of all such respiratory processes. This result has been achieved in the following series of experiments.

Experiment.

The enzyme. Larvæ of *Attagenus undulatus* were collected in quantity by placing folded sheets of brown paper in masses of infected grain, the larvæ either to avoid the light or for the sake of warmth, collected in the folds on the underside of the paper. 192 grams of the larvæ, separated and collected in the afternoon were allowed to stand overnight in a beaker. On the following morning it was noticed that a considerable quantity of moisture was deposited on the upper portion and on the cover of the beaker. It was also noticed that these larvæ in mass had a temperature of 22·1°C. On exposure to fresh air and transference to a second beaker the temperature of the larvæ rose from 22·1°C to 28·9°C in the course of 15 minutes, indicating a

[1] Biedermann. *Pflüger's Arch.* **72**. 105. 1898.
[2] Von Furth and Schneider. *Hofmeister's Beiträge* **1**, 229. 1901.

rapid reaction with the fresh oxygen with which they had been brought in contact in their transfer from the beaker in which they had been stored overnight.

The above quantity of larvæ was mixed with an equal weight of fine sand and reduced to a pulp, chloroform water being added from time to time during the process of grinding. In all 22 c.c. of water were added. The mixture was allowed to stand for one hour at room temperature, and then strained through a fine cloth. The filtrate measured 170 c.c. and was of a grey colour and was fairly thick. 250 c.c. of 95% alcohol were then added and the precipitate formed. separated by filtration through a Büchner funnel. The precipitate was taken up with chloroform water, filtered, and again precipitated with 95% alcohol. The second precipitate was washed with a small quantity of water to remove the alcohol and then suspended in 100 c.c. of water and covered with a layer of toluene and placed in a stoppered bottle.

A second solution was prepared using only chloroform water to extract the powdered larvæ. No precipitation with alcohol was here resorted to. These two solutions were used to test for *tyrosinase*. Pure tyrosin prepared by Fischer's method from silk by acid hydrolysis was recrystallized from water, and aqueous solutions of 0·05%, 0·1% and 0·5% strength prepared. The action of the enzyme was tested by adding to 5 c.c. of the above solution contained in a test-tube 5 c.c. of the enzyme solution, closing the tube with a cork and maintaining it at a constant temperature of 30°C. After periods varying from 1 to 18 hours a dark bluish black colour is developed in the upper layer of liquid (nearest the air). This colour was more intense in the case of the 0·1% solution of tyrosin and with the unprecipitated enzyme solution.

The action of the enzyme was then studied on (1) eugenol (2) carvacrol (3) toluidine (4) o, m & p. xylene (5) phenol-phthalin (6) thymol.

2% solutions of these substances were prepared in alcohol of 20% strength (except phenol-phthalin which was of 0·25% strength in 50% alcohol). 5 c.c. of the test and 5 c.c. of the enzyme solution were as before placed in a corked tube, shaken, and placed in a constant temperature bath at 30°C. for 18 hours. All these substances showed signs of oxidation, but it was more developed in the case of the three xylenes.

In the case of the enzyme solution prepared without alcoholic precipitation, a slight black colour itself developed which grew more marked after the lapse of some time. This indicates the presence of tyrosin as well as tyrosinase in the body of the weevil, both of which substances pass into solution

in chloroform water, the amount of tyrosin is evidently very small. In order to test the hypothesis that ozone or nascent oxygen is or may be present in the cell during active oxidation, a solution of tyrosin mixed with enzyme solution was subjected to the action of ozone. If the hypothesis were correct we should expect more rapid oxidation of the tyrosin by this means. As a matter of fact no colour was developed, the enzyme being evidently destroyed by even traces of ozone. The action of the enzyme was completely inhibited by boiling the solution.

Ether also appears to destroy the enzyme. An attempt was made to prepare a solution of the enzyme after removal of the fat by ether. The crushed larvæ mixed with sand were first extracted with ether to remove fat and afterwards with chloroform water. The aqueous extract so prepared failed to bring about the oxidation of tyrosine. Either the enzyme had been destroyed or was entirely removed in the ethereal washing.

Influence of acid or alkali on the activity of the enzyme.

The aqueous extract of the crushed larvæ was found to be faintly acid to litmus, this we should expect as partial oxidation of the fats would take place in the process of preparing the solution, with the formation of acid bodies. The solution so prepared was found to give but a slight reaction towards tyrosin while in this acid condition. Rendering the liquid faintly alkaline with either ammonia or sodium carbonate induced a much more rapid reaction, ammonia being more effective than sodium carbonate.

Consequently in all subsequent tests made for comparison the enzymic solutions were rendered faintly alkaline with ammonia. Abderhalden and Guggenheim[1] showed the necessity of using alkaline solution in working with tyrosinase. These authors used sodium carbonate of 0·04% strength, tyrosin of 0·05% strength in working on *tyrosinase*.

In the higher animals there is no doubt that alkaline phosphates are always present in the plasma and these alkalies play an impoitant part in regulating the amount of carbon dioxide held in solution. The protein substances are actually present in some cases as alkaline salts in the serum, and as the CO_2 concentration increases it undoubtedly replaces the protein in

[1] Abderhalden and Guggenheim. *Zeit. für Physiolog. Chem.* **54**, 331.

its combination with the alkali[1]. The oxidase enzymes then are probably adjusted to work under alkaline rather than under acid conditions and this observation of ours confirms that of Abderhalden and Guggenheim.

EXPERIMENT.

Effect of starving the larvæ with a limited supply of air.

Fifty grammes of larvæ were placed in the incubating tube of the apparatus described on page 231 and the gases of the tube analysed at the end of twenty-four hours, and again after three or four days. The following table shows the effect of the treatment.

TABLE XXXII.

No. of experiment	Date of charging the tube	Date of analysis	Carbon dioxide %	Oxygen %
Experiment 1	18-3-1914	19-3-1914	24·8	1·08
		23 3 1914	26·4	0·60
Experiment 2	27-3-1914	28 3-1914	25·0	1·10
		31-3 1914	26·6	1·09

We expected to find that as the oxygen of the air present in the incubation tube became exhausted the larvæ would die from asphyxiation, the actual lethal period varying according to the "condition" of the larvæ. But it is clear from the above figures that the process of respiration has continued after the exhaustion of the supply of atmospheric oxygen.

Taking the composition of the air by volume to be 21% oxygen and, 79% nitrogen and other gases, we see that the amount of carbon dioxide produced in excess of the atmospheric oxygen used is from 4·8% to 6·6%. For we see from the above figures that the last 1% of atmospheric oxygen present in the tube has not been available for respiration—in other words, the animal has not been able to make use of this—but that after the removal of 20 out of the 21

[1] Serloti. Hoppe-Seyler. *Medizin. Chem. untersuch. Berlin*, 1868.
„ *Pflüger's. Arch.* **58**, 511. 1894.
„ *die Kohlensäure des Blutes*, p. 11, Bonn, 1864.
„ *Memoir de l' Acad. de St. Petersburg*, **26**, 60. 1879.

volumes of oxygen present (that is 95% of the oxygen originally present) the larvæ fall back on an internal oxygen supply, and respiration continues with the production of a further 5% or 6% of carbon dioxide. Since there is no supply of stored oxygen in these larvæ similar to that in the blood of the higher animals we must concede that the carbon dioxide has resulted from the partial decomposition of chemical substances rich in oxygen, present in the tissue of the larvæ. Most insects in the larval stage secrete large quantities of fat which serve them as a reserve food-supply during the pupal stage. We therefore analysed the larvæ of *Attagenus undulatus* both before and after starving in an enclosed space similar to the above experiment. In this method 50 grams of larvæ were taken for analysis, crushed with excess of sand, and extracted with ether in a Soxhlet's apparatus, and the amount of fat in the ethereal extract determined.

TABLE XXXIII.

Effects of starvation in an enclosed atmosphere on the fat contents of the larvæ of Attagenus undulatus :—

Percentage of fat in the fresh larvæ	28·73%
Percentage of fat present in the same larvæ starved for 5 days	26·3%

The time at our disposal did not allow us to investigate the exact chemical nature of the fats, nor is this essential to the enquiry. These will probably consist for the most part of glycerides of the saturated fatty acids, and both the trihydric alcohol glycerine $CH_2OH.CHOH.CH_2OH$ and the fatty acids combined with it ($C_n.H_{2n+1}.COOH$) can under suitable conditions be made to yield a supply of energy by partial reduction, with the formation of carbon-dioxide and carbon compounds richer in carbon and hydrogen, or even of hydrogen itself, as is the case in the anaerobic fermentation of cellulose.

In all probability hydrolysis of the fat first takes place with the formation of fatty acids and glycerine, and this glycerine is almost certainly the source of the energy indicated by the presence of the excess of carbon dioxide produced, as in the reduction of the higher fatty acids a small amount of carbon-dioxide would be accompanied by the production of relatively large quantities of hydrocarbons which could not have escaped notice. In the experiments

stated above large quantities of hydrocarbons or of hydrogen were not produced though traces were found.

The next step was to obtain proof that the reactions noted above were enzymic. In order to do this it was merely necessary to destroy the larva as a living organism but under such conditions as would leave the enzymic contents of the tissue unimpaired. If respiration is then a result of enzymic action, either simple or complex, we should obtain an absorption of atmospheric oxygen, and after this had all disappeared, a further production of carbon-dioxide from the breaking down of the hydrolysed fats present.

Experiment. Varying quantities of fresh larvæ were reduced to a fine powder with excess of sand (recently ignited and cooled to destroy any organic matter, bacteria, etc.), the amount of sand added being regulated so as to yield a fairly dry granular mass after grinding.

A little chloroform (and in another experiment toluene) was added to the mass in grinding to render this sterile. The incubation tube described above was then sterilized with chloroform vapour (or toluene) and the sand-larval mass introduced. Chloroform vapour was now passed through the whole apparatus for 20 minutes to render the incubation tube and its contents sterile, and this vapour was afterwards removed by a current of sterile air passed for from 20 to 30 minutes. The tube was then closed and kept at a constant temperature of 30°C and the gases of the tube analysed from time to time. The precautions taken to ensure the sterility of the tube and its contents were to prevent any bacterial changes taking place in the crushed material. A control experiment was done subjecting the mass to steam heat in an autoclave for 20 minutes at 115°C and as this failed to destroy the enzyme a second control was done destroying the enzyme by subjecting it to a temperature of 125° C. for 30 minutes in the autoclave.

In connection with the difficulty here experienced of destroying the "oxidase" by steam heat we would cite Wood's[1] experience in the case of tobacco oxidase; he found this enzyme to be very refractive to destruction by heat. Moore and Whitley[2] also refer to the difficulty of destroying vegetable "peroxidases" by boiling their solutions and cite that potato juice may be boiled for half a minute without accomplishing this destruction.

[1] Wood. *Bull. U. S. Dept. of Agriculture* No. 18, p. 17.
[2] Moore and Whitley. *Bio-Chem. J.* Vol. IV, 1909, p. 139.

The following table gives the results obtained :—

TABLE XXXIV.

Analysis of gases expired by sterile crushed larvæ of A. undulatus with a limited supply of air at a temperature of 30°C.

Experiment	Time of incubation in hours	Carbon-dioxide %	Hydrogen %	Methane %
(A) 7·86 grams of larvæ sterilized with toluene	18 hours	11·3	0·14	...
	30 ,,	21·18	2·3	2·4
	45 ,,	21·7	1 7	2·0
(B) 9·47 grams of larvæ sterilized with chloroform.	18 hours.	22·7	2·05	0·98
	30 ,,	24·69	0·65	1·7
	45 ,,	24·8	0·85	0·97
(C) 17·05 grams of larvæ sterilized with chloroform.	18 hours.	20·8	1·03	1·54
	30 ,,	29·2	1·17	1·94
	45 ,,	34·02	1·92	2·26
(D) 5·68 grams of larvæ sterilized with chloroform.	18 hours.	12·8	0·74	1·8
	30 ,,	not analysed.	not analysed	...
	45 ,,	22·1	1·02	1·9
(E) Control 16·2 grams of larvæ sterilized in steam autoclave for 20 minutes at 115°C.	18 hours.	0·66	0·8	0·6
	30 ,,	1·9	0·9	1·1
	45 ,,	8·0	1·0	1·06
(F) Control 6·4 grams of larvæ sterilized in the steam autoclave for 30 minutes at 125°C	18 hours.	0·07	0·6	0·8
	30 ,,	not analysed.	...	not analysed.
	45 ,,	0·2	0·58	1·9

It is very clear from these figures that the chemical processes of respiration as indicated by the absorption of oxygen and the production of carbon-dioxide have gone on in the larvæ after death in an exactly similar manner to those in process during life. It is in fact respiration after death and the proof that the action is enzymic is shown in the analyses of the gases in experiments E and F where the enzyme has been partially or entirely destroyed by steam heat. The importance of this result cannot be too greatly emphasised both in its theoretical and practical aspect. It disposes of all the older theories regarding the mechanism of the respiratory process and proves beyond doubt that this is enzymic. It meets the demand of the physiologist that respiration is really a cell function no matter how or by what means the oxygen

actually reaches the cell plasma or whether the cell is vegetable or animal and that the actual oxidation of the nutrient present there, is under the control of the cell protoplasm. The presence of oxidases in plant tissue indicates that respiration in the vegetable cell is the same in all its essential features as that in the animal cell, but the greater demands of the latter for energy result in a larger display of the chemical processes attendant on respiration. It is in such members of the vegetable kingdom as exhibit abnormally rapid growth like the fungi, that we find these oxidases in greatest abundance, and their presence there evidently indicates that respiration is proceeding at a rapid rate. The change in colour of these bodies after death is similar to the phenomenon of melanosis in the insects, and there, as in the case we have examined, we shall no doubt find that respiration proceeds after the death of the plant, using the term death to cover the disintegration of the plant as a whole but not the destruction of the enzymes.

It is well known that aerobes placed in an atmosphere of hydrogen continue to produce carbon-dioxide until death ensues, and that even when placed in a vitiated atmosphere containing only 3% or 4% of oxygen a volume of carbon-dioxide is evolved which greatly exceeds the amount of oxygen consumed. Stich has shown that in such cases the plant accommodates itself to the changed circumstances and after some time the diminished oxygen supply suffices for the diminished respiratory activity.

What is of equal importance it has been observed by many workers that in the so-called intramolecular respiration of plants reduction products of the sugars such as alcohol and hydrogen in addition to carbon-dioxide have been found.

Pfeffer states that the intramolecular respiration of plants is not directly connected with the decomposition of protein substances but he overlooks the fact that it may have a very important bearing on the vitality of the embryo in throwing the whole burden of supplying the necessary energy to maintain the vital processes on the internal cleavage of sugar and protein substances as well as intermediary compounds of these.

The resistance of aerobes to a vitiated atmosphere or to use Pfeffer's words "the intensity and character of intramolecular respiration" is dependent upon the specific nature of the plant and upon the quality and quantity of the available food materials.

Reference may be made to Pfeffer's *Physiology of Plants*, volume 1, page 536, *et seq.*, where a bibliography of the original papers dealing with the respiration of plants is given.

The next important step in proving the function of these oxidases in animal tissues is to examine their action on the purin bases and particularly their action on xanthin. This has already been done by Von Burian and by Wiechnowski and H. Wiener.[1] These authors obtained an enzyme capable of oxidising xanthin to uric acid by extracting the finely powdered organs with a 0·05% solution of sodium carbonate. We hope to repeat this work on *A. undulatus* when the larvæ are again available in quantity.

We may now venture to interpret our previous results and observations in the light of these latter discoveries.

Taking first the case of carbon-dioxide and its effect on the larvæ of *A. undulatus* we have already noted that on first introducing the larvæ into the gas a short period of intense activity extending over only a few seconds, was followed by a lengthy period of coma varying from 63 hours in one experiment at 30°C to 87 hours in another at the same temperature. When first introduced into the gas, the tracheal system of the insect is not uniformly charged with air. In the case of man it has been shown that it is only the composition of the air in the alveoli which regulates the rate of gaseous exchange between the outer air and the blood gases[2] [3] and so in the insect it will be this composition of the air in the finer capillaries of the tracheal system which will come into play. Evidently then the first effect of a decreased carbon-dioxide exchange from the insect to the outer air due to the rapidly increasing concentration of carbon-dioxide in the tracheal system, will be instantly followed by muscular effort both of the respiratory muscles of the trachea in their increasing demand for air, and of the whole insect to escape from an undesirable environment. In a very short time, however, the insect is cut off from gaseous oxidation and all muscular activity ceases. It now enters on a more or less lengthy resting period from which, if not restored to air at a suitable temperature, it will ultimately and imperceptibly pass the boundary between life and death. Since it is clear that during this period the organism is quite cut off from all external supplies of oxygen in the gaseous form, and since any oxygen present in the insect will be rapidly used up or passed by diffusion into the outer atmosphere of carbon-dioxide in the trachea, and as we have shown above the insect is incapable of using more than 95 per cent. of the atmospheric oxygen when the air is replaced by carbon-dioxide, it must be making use of chemically combined oxygen to maintain the life processes during the period of coma which precedes death.

[1] Von Burian, Wiechnowski and Wiener, *Hof. Beiträge* **9**, 247 and 295, 1903.
[2] Wolffberg. *Pflüger's Arch.* **4**, 465, 1871, and **6**, 23, 1872.
[3] Nussabaum. *Pflüger's Arch.* **7**, 296, 1873.

This energy as we have already seen is derived from the reserve food in the form of fats, and is rendered available by an oxidase enzyme. The wide difference between the lethal periods for carbon-dioxide at different periods of the year shown in graphic form in Figure 4 as well as the difficulty experienced in obtaining concordant result in the same experiment with all the insects (*see* Table XXVII, page 234) indicate that the personal factor in each insect is regulated by the activity of its enzymic contents at the time of the experiment. Since these enzymes then play the part they seem to do from these observations described above, their presence is clearly connected not only with the process of respiration but in some modified form controls the supply of energy from the internal combustion of fats which must take place when the animal is cut off from air, either artificially as in the above described experiment where we have placed it in an atmosphere of carbon-dioxide or other gas, or in the natural state during the pupa and metamorphosis stage in which there appears to be no provision for ordinary respiratory processes. A parallel case to this may be cited in that of the higher animals which hibernate. Such animals are well-known to conserve a considerable amount of reserve food in the form of fat; issuing from their winter quarters they are invariably lean and most or all of the fat has disappeared.[1] In these cases we know that respiration continues at a slower rate, but it may be helped by internal respiration. We (the authors) have no information regarding this process and in the light of our results it appears to offer a fertile field for enquiry on similar lines. It will be remembered that we have shown carbon-dioxide to have an injurious effect on the germinating power of wheat, and we have not had time to examine the comparative effect of hydrogen and nitrogen on this value in addition to that of carbon-dioxide but respiration in the embryo of the grain is no doubt carried on in exactly the same manner by means of enzymes, hence the deleterious effect of carbon-dioxide in cutting off the supply of atmospheric oxygen and so allowing the embryo a less supply of energy from the nutrient foods contained in it, *viz.*, only the energy available from the cleavage of the food substances.

We are at present unable to offer a complete explanation of the shorter lethal period in the case of the gases of less density. The laws of gaseous diffusion must apply, and be in part responsible for the shortening of the period in the case of gases lighter than carbon-dioxide, for in the production of carbon-dioxide by the cleavage of compounds rich in oxygen such as evidently takes place in the larvæ of *Attagenus undulatus*, the rate at which this will

[1] Carlier, E. W., and Evans, C. A. L. *Journal of Anatomy and Physiology*. Vol. XXXVIII, pp. 16—31.

diffuse out *viâ* the tracheæ into the outer atmosphere, must be greater, as this atmosphere is made one of less density, and thus far our results confirm Graham's supposition. But this is not all, for we have seen that no numerical relationship can be established between the rates of diffusion of the different gases and the length of the lethal period.

The shortening of the lethal period with increase of temperature, proceeding as it does in a regular manner in some of the cases examined, indicates this period is in all probability a function of a chemical reaction and obeys the laws governing chemical reactions. This is no doubt true since both hydrolysis and cleavage as the result of enzymic activity will proceed at an increasing rate with the rise in temperature until the optimum temperature for the working of the enzyme is reached, after which the curve will break away to the ordinate. This point evidently has not been reached in our experiments with carbon-dioxide, hydrogen, or nitrogen. More rapid hydrolysis and cleavage will result in a more rapid production of carbon-dioxide, a more rapid consumption of the available food, and a general tendency to shorten the coma stage or lethal period. Thus far we seem justified in interpreting the results obtained with the effect of the gases carbon-dioxide, hydrogen and nitrogen on the beetles *A. undulatus*, *R. dominica*, and *C. oryzæ*.

SUMMARY.

This portion of the enquiry has proved the process of respiration to be the result of enzymic activity under the control of the cell and experimental evidence in support of this is produced in separating the particular type of oxidation which takes place in the larvæ of *Attagenus undulatus* from the living processes.

The inert gases carbon-dioxide, hydrogen, and nitrogen, have been shown to have very different lethal periods, and the lighter or less dense the gas, the shorter the time taken to kill the insect.

Increase in temperature also brings about a shortening of the lethal period. Respiration being shown to be an enzymic action the ordinary chemical laws governing the rate of chemical reaction will apply, increasing velocity of reaction with increasing temperature.

Gaseous diffusion also plays some part here in the less dense atmosphere hastening the diffusion of the carbon-dioxide produced by internal respiration, and so accelerating the consumption of the available food. The lethal

periods do not coincide with the diffusion ratios calculated from the gas densities.

We are forced to the conclusion that no inert gas (such as carbon-dioxide) can be economically used as an asphyxiating agent for these insects, owing to their ability to enter on a "hibernating" stage when atmospheric oxygen fails them and also because carbon-dioxide itself materially affects the germinating power of wheat. We must therefore turn to the use of chemical deterrents or mechanical methods of treatment.

CHAPTER V.

THE EFFECT OF MOISTNESS AND DRYNESS.

This subject has been dealt with by several writers, and a summary of the information on the subject is given by Noël Paton,[1] who quotes the conclusions arrived at by Fletcher. The most important is number three which states :—"Whilst containing less than 8 per cent. (moisture) stored wheat is immune from attack by weevil, and any weevils which may obtain access to it are soon killed off."

From this Noël Paton was led to conclude that desiccation would be an effective means for preserving stored grain, and goes so far as to say :— "But in view of the facts demonstrated by Messrs. Fletcher and Leather the probability is that the elevators will have the effect desired, not through employing gas of any kind but through the operation of their drying appliances."

This conclusion is, however, premature, for the experiments carried out by Fletcher were with *C. oryzæ* only, but by the use of the term "weevil" in the conclusion drawn from the experiments, a wider significance is conveyed to the lay mind than the experiments warranted, since all insects attacking wheat are "weevils" to the uninitiated.

As has been previously pointed out, the insects which have to be dealt with in the Punjab are three in number, one being *C. oryzæ* and it was necessary to know whether the other two *A. undulatus* and *R. dominica* behaved in the same way towards dryness as *C. oryzæ* had been shown to do.

Two series of experiments have been carried out, each of a different nature.

Whilst at Pusa one of us (A. J. G.) carried out a series of tests in collaboration with Leather on similar lines to those conducted by Lefroy and Fletcher, but dealing with each of the insects concerned separately. The experiment was divided into two sets, in the one, glass stoppered bottles were used and in the other corked tubes sealed with paraffin wax. The vessels were divided into series and were filled with wheat containing roughly the following percentages of moisture, 5%, 7%, 10% and 15%, and into each

[1] *Loc. cit.*

vessel 20 live insects were placed. In the case of *C. oryzæ* and *R. dominica* adult beetles were used, but with *A. undulatus* larvæ only, as adults were not obtainable (it was not known at the time that these experiments were conducted that it was only in the larval stage that this insect damaged the wheat). The wheat which was placed in the stoppered bottles was fumigated with carbon-bisulphide before being moistened or dried so as to bring its moisture content to the required amount, but that which was used in the tubes was not fumigated but had been carefully examined and selected. The reason for this was, that in some preliminary experiments with fumigated wheat, the insects had all died, and it was thought that perhaps the fumigant may have been responsible for this. For each percentage of moisture there were three bottles and three tubes for each species, so that the experiment was done in triplicate in each set.

The series in the glass bottles were started on 22nd October 1913 and those in the tubes on 24th November. Both remained undisturbed in the laboratory until 1st December when, the temperature falling below what was considered desirable, they were placed in a large constant temperature chamber at 30°C., where they remained until January 1914. All the vessels were opened and examined between 16th and 23rd January, and the results obtained are given in the following tables in which the numbers 1, 2 and 3 refer to the stoppered bottles containing fumigated wheat, and 4, 5 and 6 refer to the paraffined corked tubes containing unfumigated wheat in each case.

TABLE XXXV.

A. undulatus.

No.	Percentage of moisture	No. of insects put in	No. of insects found	No. of insects alive	No. of insects dead	REMARKS
	%					
1	5	20	19	..	19	3 cast skins.
2	5	20	19	10 (1)	9(2)	(1) 8 larvæ, 2 beetles. (2) 3 larvæ, 6 beetles. 45 cast skins.
3	5	20	16	..	16	3 cast skins.
4	5	20	20	...	20	
5	5	20	23	17(3)	6(4)	(3) 15 larvæ, 1 pupa, 1 beetle. (4) 2 larvæ, 4 beetles, 36 cast skins. Of the live larvæ 4 were very small and had evidently hatched from eggs.
6	5	20	18	16(5)	2(6)	(5) 16 larvæ. (6) 1 larva, 1 beetle, 42 cast skins.

TABLE XXXV—*contd.*

A. undulatus—concld.

No.	Percentage of moisture	No. of insects put in	No. of insects found	No. of insects alive	No. of insects dead	REMARKS
	%					
1	7	20	18	..	18	5 cast skins.
2	7	20	19	14(7)	5(8)	(7) 8 larvæ, 1 pupa, 5 beetles. (8) 5 beetles, 63 cast skins. Eggs found.
3	7	20	19		19	38 cast skins
4	7	20	17	16(9)	1(10)	(9) 15 larvæ, 1 beetle. (10) 1 beetle, 39 cast skins.
5	7	20	20	...	20	4 cast skins.
6	7	20	19	...	19	
1	10	20	16	..	16	2 cast skins.
2	10	20	17	..	17	13 cast skins.
3	10	24	19	...	19	6 cast skins.
4	11	20	15	13(11)	2(12)	(11) 9 larvæ, 4 beetles. (12) 2 larvæ, 2 beetles. 38 cast skins.
5	11	20	17	...	17	
6	11	20	14	3(13)	11(14)	(13) 3 larvæ. (14) 11 larvæ, 19 cast skins.
1	12	20	17	..	17	9 cast skins
2	12	20	11	5(15)	6(16)	(15) 5 larvæ. (16) 6 larvæ. 32 cast skins.
3	12	20	19	...	19	5 cast skins.
4	12	20	16	15(17)	1(18)	(17) 14 larvæ, 1 pupa. (18) 1 larva, 30 cast skins. An infection of *C. oryzæ* had occurred.
5	12	20	20	...	20	
6	12	20	18	...	18	
1	15	20	20	...	20	
2	15	20	17	...	17	
3	15	20	19	...	19	3 cast skins.
4	15	20	18	...	18	
5	15	20	20	...	20	
6	15	20	17	12(19)	5(20)	(19) 12 larvæ. (20) 5 larvæ, 30 cast skins. An infection of *C. oryzæ* had occurred.

TABLE XXXV—*contd.*

R. dominica.

No.	Percentage of moisture	No. of insects put in	No. of insects found	No. of insects alive	No. of insects dead	REMARKS
	%					
1	5	20	17	3	14	
2	5	20	20	...	20	
3	5	20	20	...	20	
4	5	20	20	...	20	
5	5	20	19	...	19	
6	5	20	18	16	2	Several eggs found.
1	7	20	18	16	3	
2	7	20	20	...	20	
3	7	20	20	...	20	
4	7	20	21	14	7	
5	7	20	19	...	19	
6	7	20	20	15	5	
1	10	20	28	12	16	
2	10	20	20	...	20	
3	10	20	20	...	20	
4	11	20	20	...	20	
5	11	20	20	..	20	
6	11	20	20	14	6	
1	12	20	26	23	3	
2	12	20	20	...	20	
3	12	20	19	...	19	
4	12	20	20	...	20	
5	12	20	19	...	19	
6	12	20	18	...	18	
1	15	20	20	9	11	
2	15	20	20	...	20	
3	15	20	21	...	21	
4	15	20	20	...	20	
5	15	20	20	...	20	
6	15	20	17	...	17	

TABLE XXXV—*concld.*

C. oryzæ.

No.	Percentage of moisture	No. of insects put in	No. of insects found	No. of insects alive	No. of insects dead	REMARKS
	%					
1	5	20	21	...	21	
2	5	20	20	...	20	
3	5	20	22	...	22	
4	5	20	20	...	20	
5	5	20	20	...	20	
6	5	20	20	...	20	
1	7	20	21	...	21	
2	7	20	22	...	22	
3	7	20	21	...	21	
4	7	20	19	...	19	
5	7	20	19	...	19	
6	7	20	24	...	24	
1	10	20	20	...	20	
2	10	20	21	...	21	
3	10	20	44	17	27	
4	11	20	25	...	25	
5	11	20	25	...	25	
6	11	20	23	...	23	
1	12	20	20	...	20	
2	12	20	25	...	25	
3	12	20	44	12	32	
4	12	20	20	...	20	
5	12	20	22	...	22	
6	12	20	20	...	20	
1	15	20	23	...	23	
2	15	20	20	...	20	
3	15	20	27	...	27	
4	15	20	20	...	20	
5	15	20	20	...	20	
6	15	20	20	...	20	

An examination of these results shows that although wheat containing less than 8 per cent. moisture is immune from the attacks of *C. oryzæ* (which is the form in which Fletcher should have stated his conclusion), yet with *A. undulatus* and *R. dominica* a very different state of affairs exists. In the case of *A. undulatus* the very reverse is the case, in that, although some of the insects had survived in each of the various percentages of moisture, yet breeding had taken place more frequently in the lower percentages, oviposition apparently having occurred in the cases of 5 per cent. and 7 per cent. moisture. With *R. dominica* some insects had survived under each of the conditions, but it is noticeable that breeding had gone on even with only 5 per cent. moisture. The experiment is open to criticism from the point of view that our knowledge of the condition of the atmosphere inside the bottles is very limited, and the results may have been disturbed by the introduction of other factors, especially in the high percentages of moisture, produced by partial germination or fermentation in the wheat. It was therefore deemed advisable to test the matter in another way.

The second series of experiments was carried out at Lyallpur on quite different lines. The object of the experiment was to determine the length of time which the insects would live without food and at different temperatures in, on the one hand, an atmosphere saturated with moisture, and on the other hand an atmosphere entirely free from moisture. The results obtained could then be plotted in the form of a curve and the space between the "dryness curve" and the "moistness curve" should be a measure of the sensitiveness of each of the species to moistness or dryness. The insects were confined in test tubes of the shape shown at A in Fig. 7 their activities being restricted to the closed end of the tube by means of the wire gauze plug. In the body of the tube a smaller tube was placed

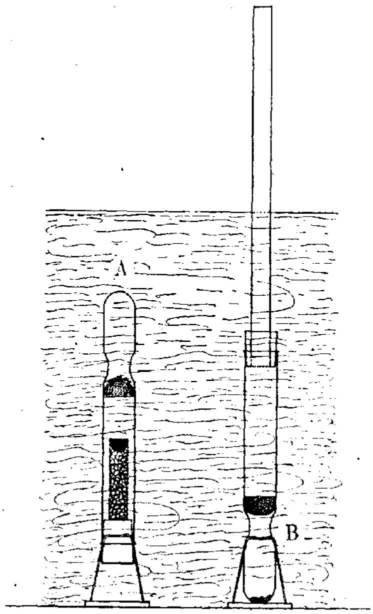


Fig. 7.

which contained the reagent used to produce the atmosphere required, and the whole was sealed by a rubber bung. Each tube, rounded end upwards, was

R. dominica.

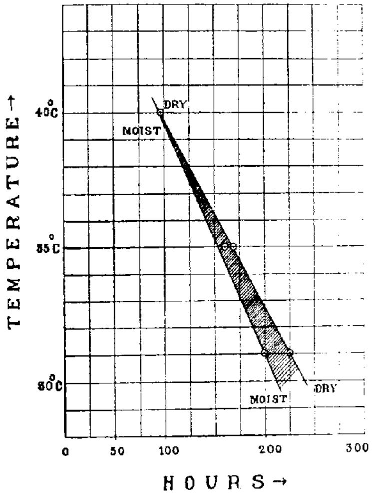


Fig. 8.

A. undulatus.

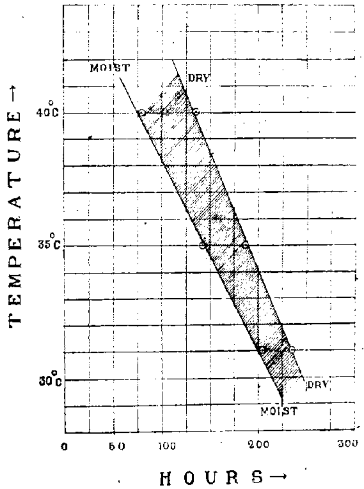


Fig. 9.

C. oryzæ.

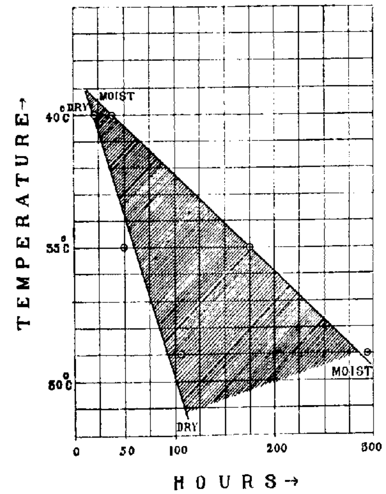


Fig. 10.

then submerged by means of the leaden sinker in a tank of water kept at a constant temperature. In order to produce an atmosphere saturated with moisture, the reagent tube contained small pieces of pumice soaked with a dilute solution of caustic potash, the use of the potash being to absorb any carbon-dioxide which might be given off by the insects; and the dry atmosphere was produced by the use of fused calcium-chloride, which however was also mixed with soda lime to absorb any carbon-dioxide. The control tube used with each of the experiments is shown at B, Fig. 7, the wide

tube piercing the bung being open to the atmosphere above the water in the tank. In carrying out the experiment four moist tubes and four dry tubes were used for each species and each tube charged with ten insects. The control tube was charged with twenty insects. The experiment was carried out at temperatures of 31°C, 35°C and 40°C. The chief difficulty met with was to determine exactly when the insects were dead, for it was found that often when to all appearances death had occurred, the insects on removal from the tube would recover. In practice therefore tubes in which the insects had all apparently succumbed were left for varying periods before they were removed, and in this way a number of periods was obtained some of which were too short and some too long. An average of these figures will give approximately the period fatal under the particular conditions, and the periods given in Table XXXVI are such averages. These were plotted in the curves shown in Figs. 8, 9 and 10 the lethal periods being plotted as ordinates and the temperatures as abscissæ.

TABLE XXXVI.

TEMPERATURE	LETHAL PERIOD IN HOURS					
	A. undulatus		*R. dominica.*		*C. oryzæ.*	
	Moist	Dry	Moist	Dry	Moist	Dry
31°C ...	205	235	199	223	295	80
35°C ...	143	188	162	167	173	50
40°C .	78	134	97	98	38	22

An examination of the curves shows that in the case of *C. oryzæ* the dryness curve is steeper than, and is on the left hand side of the moistness curve, and the space between the two curves is considerable. With *R. dominica* the dryness curve is on the right of the moistness curve, but the space between them is very small, and for *A. undulatus* the dryness curve is on the right hand side of the moistness curve and the space between them is more than that of *R. dominica* but less than *C. oryzæ*.

The deduction which can be drawn from this is that, whereas dryness has a marked inhibitory effect upon *C. oryzæ*, it has practically the same effect as moistness on *R. dominica* and no effect on *A. undulatus*, this insect in fact preferring dry conditions to moist ones. This supports the results obtained in the first experiment.

It is clear therefore from these experiments that desiccation is not a remedy which is effective against all the insects which attack stored wheat in the Punjab, though it is so in the case of *C. oryzæ*. *R. dominica* seems to be practically indifferent to either moistness or dryness and *A. undulatus* seems to prefer dry conditions and moisture in excess appears to inhibit its activities.

The results are supported by other investigations. If reference be made to the Tables VII, XII and XV, which give the length of life-history of these insects at different periods of the year, it will be seen that the shortest periods for *A. undulatus* are during May, June and the first half of July which are the hottest and driest months of the year. In the case of *R. dominica* the effect of the moist conditions is not so noticeable, the length of life-history being more influenced by temperature. The case of *C. oryzæ* is remarkable, for breeding ceased entirely during the hot dry months of May, June and July and it was not until the moisture conditions had been made sufficiently favourable by the advent of the rains, that development took place.

The distribution of these insects in the Punjab gives further support to these results for if Table I, pages 169 and 170, are consulted, it will be seen that in the drier places such as Sirsa and Lyallpur, where the rainfall is small, *A. undulatus* is the commonest insect found, while in Amritsar, Gurdaspur and places in that district which are closer to the foot hills and have consequently a much higher rainfall, *C. oryzæ* predominates.

CHAPTER VI.

Remedial measures and experiments connected with them.

1. *Chemical Deterrents.*

The use of Chemical Deterrents. An account has been published by one of us (A. J. Grove)[1] on the use of naphthalene in preventing insect attacks on stored maize. It was shown that the results obtained by its use were as good as those obtained by fumigation with carbon bi-sulphide and that the germination of the grain was not affected.

In a province where such a large quantity of wheat is grown like the Punjab the preservation of the wheat which is kept for seed is a serious problem and experiments were started to see whether the use of naphthalene could be applied to wheat in the same way in which it has been applied to maize.

The first point was to determine whether the germination capacity of the wheat was affected in any way by the naphthalene.

In the case of the maize the proportion of naphthalene used was half a seer (1 lb.) per twenty-five maunds (2,000 pounds) or 0·05%. In the experiments with the wheat varying percentages were used. The wheat used was first fumigated with carbon bi-sulphide to ensure that no grain would be spoiled by insect attack and then divided into lots of 300 grams each and these were placed in glass stoppered bottles with the following quantities of naphthalene :—Nil (to act as a control); 2·5 grams; 5 grams; 10 grams; 15 grams; and 20 grams being 0·8 per cent.; 1·6 per cent.; 3·3 per cent.; 5 per cent.; and 6·6 per cent. The experiment was started on 16th December 1912 and on the 17th of each subsequent month until December 1913, 200 grains of wheat were taken from each and placed in a dish upon damp blotting paper to determine the percentage of germination. The results obtained are given in Table XXXVII.

[1] Grove, A. J. Some Experiments with Maize stored in bins. *Agricultural Journal of India*, Vol. IX, pp. 92—98, 1914.

TABLE XXXVII.

Quantity of naphthalene to 300 grains of wheat	Number of wheat grains which germinated out of 200.												
	January	February	March	April	May	June	July	August	September	October	November	December	REMARKS
Nil ...	175	173	184	176	183	177	180	191	178	181	180	187	
2·5 grams	181	183	167	176	172	168	161	162	173	152	134	175	
5 ,,	178	176	180	175	171	162	168	172	172	156	126	168	
10 ,,	181	184	170	166	140	161	166	172	167	154	131	160	
15 ,,	177	164	172	176	138	167	170	175	168	149	123	174	
20 ,,	180	182	173	168	144	162	168	176	171	175	122	171	

These figures show that for the first nine months, that is, from January to September, there is no falling off in the percentage germination that would not be included in the errors of the experiment, and curiously enough the figures obtained in December were higher than those obtained in October and November, and compare very favourably with those of the earlier months. The explanation of this is that in October and November the laboratory temperature was so much lower than in the latter months that the naphthalene did not evaporate so easily from the grains, and the vapour accumulating in the germinating dishes (a distinct smell of naphthalene was observed on several occasions in the dishes), the germinating grains were injured. In December 1913 therefore the grains before being placed in the dishes were exposed to the sun for a period of six hours so that any naphthalene present might evaporate and the results obtained are therefore proportionally higher. In December 1914 that is after the wheat had been with the naphthalene for two years, a test in the field was made. 200 grains from each bottle, after exposure in the sun for several hours, were sown. Unfortunately owing to a canal closure the germinating wheat could not be irrigated by flow irrigation and the hand irrigation resorted to proved unsatisfactory and the germination on the whole was not good, but the conditions were the same for each lot of grain; the results obtained were as follows :—

Quantity of naphthalene...	nil	2·5 gm.	5 gm.	10 gm.	15 gm	20 gm.
No. of grains germinated.	36	16	28	24	25	21

From these it will be seen that even after two years the naphthalene has not had much effect upon the germinative capacity of the wheat.

Mr. H. Southern, Deputy Director of Agriculture at Gurdaspur, used naphthalene in this manner in storing his seed wheat at the Gurdaspur farm, 267 maunds ($190\frac{1}{2}$ cwt.) were stored in three large iron bins on 9th June 1914. On 11th August the writer made an examination of these bins and found no insect attack although there were a large number of insects (*C. oryzæ* chiefly) in the storehouse, in which the bins were situated. Some other wheat which had been placed in bags on the floor of the storehouse had become badly infected.

This shows that either the wheat was infected when it was stored or else became infected after storing, the latter being the most probable. It is therefore interesting to note that the wheat in the bins had remained free from insects while the wheat in the bags was infected. The cases are not quite parallel as the bins may have been instrumental in preventing the access of the insects whereas the gunny bags would not afford such protection. The method is again being tested this year.

Samples of bread made from wheat which had been stored with naphthalene :—

Since the seed wheat may be stored in the same chambers as wheat which will be sold for food, and because of the difficulty of differentiating between them in the village it was thought advisable to test the wheat after such treatment to see if sufficient naphthalene remained in the grain to flavour the flour or bread made from it. For this purpose samples of wheat treated with naphthalene were first exposed to sunlight to volatilize the naphthalene. The wheat was then reduced to a coarse flour and made into bread.

The following table gives the result of the test :—

TABLE XXXVIII.

Samples of bread made from wheat first stored with naphthalene, and afterwards exposed to sunlight before grinding :—

SAMPLE	DESCRIPTION OF THE BREAD
A. (Pusa) 10-5-1915	No smell of naphthalene.
B. ,, 10-5-1915	Slight smell of naphthalene.
C. ,, 10-5-1915	Strong smell of naphthalene.
D. ,, 10-5-1915	Slight smell of naphthalene.
E. ,, 10-5-1915	Strong smell of naphthalene.
F. ,, 10-5-1915	Slight smell of naphthalene.
(Gurdaspur) (14) 10-5-1915	Slight smell of naphthalene.
(Pusa) (12) (Gurdaspur) 10-5-1915	Slight smell of naphthalene.

It is clear from this experiment that though we may preserve wheat for seed purposes with this substance, naphthalene cannot be used for wheat which may be sold for food, owing to the objectionable taste and odour it imparts to the bread.

2. *Mechanical Treatment.*

Wheat which has been thoroughly cleaned will be less likely to suffer damage than wheat which is stored without cleaning, for the mere process of sifting will remove a large number of insects which are outside the grain and thus reduce in magnitude the factors causing damage. Moreover the removal of the dust and broken grains will reduce the available food supply for such insects as *T. castaneum*, *C. oryzæ* and *L. pusillus* which have been shown to live in the detritus and not on the entire grain. It will also reduce to some extent the number of *A. undulatus* and *R. dominica* larvæ, as it will limit their supply of broken food material and so restrict their development and ability to attack whole grains.

The mechanical cleaning of wheat is practised in the villages by the Indian shopkeeper. Practical experience has taught him that when his grain gets weevilled, it restricts the damage if he sieves out the weevils and dust, and it is therefore a common sight in the Punjab villages to see this thrifty class dealing with the problem in a manner which scientific enquiry has proved to be the best and most efficient method.

The large wheat exporting firms, too, are fully alive to the advantages to be derived from cleaning wheat before export. Though they might not prefer to have the wheat delivered to them in a clean and undamaged condition as the cleaning process is a profitable part of their business, still as one of the managing directors of a large exporting firm said to one of us in discussing the possibilities of introducing elevators into the Punjab "If you can limit the damage caused by weevils, the failure of elevators to establish themselves will be rendered more remote, more, they will probably be a success." As we have already noted in Chapter I, the actual amount of damage caused is not known. Hooper's methods of estimating the damage, mentioned in Noël Paton's *Indian Wheat and Grain Elevators*, are no doubt picturesque, but they are laboratory methods and can only give us the amount of damage under the most favourable conditions for the weevil. The actual damage is much less than Hooper's estimates and the trade figure of from 1 to 5 per cent. is much more likely to be correct since the weevils like most insects have many enemies and very rarely get the chance of doing all the damage they are capable of.

We have already shown in Chapter II the impossibility of using inflammable or poisonous gases for a large granary in this country owing to the prohibitive insurance rates which would certainly be levied, and our experiments in this memoir have shown too, the impossibility of using inert gases

as asphyxiating media, on account of the ability of the insects to protect themselves by restricted respiration.

Drying the grain we have shown in Chapters I and V to be ineffectual, as some of the insects exist better under dry than under moist conditions. *A. undulatus* and others (*R. dominica*) seem to be indifferent, and to flourish equally well in dry or moist grain. In the light of this knowledge the inclusion of a drying plant in an Indian wheat elevator seems an unnecessary expense. If included it will only be of use to deal with wheat which has suffered from exposure to rain, a comparatively rare occurrence in the Punjab.

Chemical deterrents, too, seem to present difficulties in grain which will afterwards be used for food.

We are of the opinion therefore that the only solution to the problem will be found in a mechanical separation of the insects causing the damage.

Conditions which have to be complied with in order to render mechanical treatment satisfactory.

We have shown in Chapter I that damage resulting from the three principal organisms *A. undulatus*, *C. oryzæ* and *R. dominica* is caused by the larvæ of the first and the adults of the two latter. The larvæ of *A. undulatus* start to feed almost as soon as hatched. We must therefore remove them when in a young state, or better still remove their eggs if this is possible. The adult insects of *R. dominica* and *C. oryzæ* offer no great difficulty since they can be readily sifted out. The chief difficulty seems to be presented by *A. undulatus* and the damaged grains in which weevils have hidden themselves. These grains are uniform with those of unattacked wheat and cannot be separated by sieves. We therefore turned to a mechanical method of treatment which will combine the advantage of sifting with those of a density separation. The principle on which the latter will depend will be the "floating out" of any substances which are of less density than entire or undamaged wheat. Air blasts have been used for cleaning wheat from time immemorial, the separation of the wheat from the chaff by winnowing is certainly the oldest of these methods and is still practised by the Indian peasant in very much the same manner as it must have been by his forefathers thousands of years ago. Various refinements of this method have been introduced into the machinery used for threshing and cleaning wheat, but in all of these machines that we have been able to inspect here in India sieves and shakers appear to form a necessary part of the plant. Now sieves will only operate effectively in removing impurities which are either larger or smaller than the average sized grain. By these means we can certainly clean out stones, foreign seeds of a different size,

or weevils and the detritus which result from their activity. But sieves will not remove grain which has been pierced and partially consumed if the shell is still left almost entire, nor will they remove the weevils contained in such partially hollowed grain. Such impurities not only constitute a direct loss on the entire stock, but may, if the wheat is again stored after sieving, be the starting point for fresh damage after the insects contained therein begin to breed. These grains will be lighter than undamaged wheat in a more or less marked degree according to the amount of endosperm which has been eaten, and will be moved by a current of air more easily than will undamaged grain. The size of the grains will not affect the distance they can be moved by a blast of air provided the density of the contents of the grain are fairly uniform. For though one grain may weigh less than another it will also be smaller and will present a proportionally smaller surface to the action of the air blast. If the $\left(\frac{\text{surface area}}{\text{weight of the grain}}\right)$ remains constant, the effect of the air blast will be fairly uniform, but if the denominator grows less, the effect of the blast will be to move the grain further, or to put it in another way it will require a less powerful blast to "float" the grain.

Experiment.—A mixture of damaged, undamaged wheat, weevils, and their detritus, was allowed to flow from a large glass funnel on to a smooth board sloped at an angle of about 30° (see Fig. 11). The stream of grains,

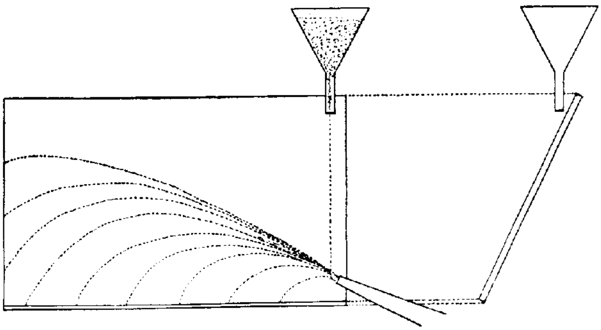


Fig. 11.

as it flows down the board, is struck laterally by a blast of air. The lightest portion of the mixture, the dust, detritus, and weevils, were deflected to the greatest degree from the original line of movement of the grain, and the undamaged grain the least deflected. Between these two extremes we find

mixtures varying from mere empty shells and light matter on the one side to slightly damaged grain on the other. This experiment proved the truth of our hypothesis in a rough manner, but this method is not directly capable of practical application, because the flow of the grain is not sufficiently uniform in character to allow of a uniform velocity in the grain before it is struck by the air blast. After a series of experiments we hit on the design, shown in Fig. 12. This separator consists of a tube (constructed in the experiments

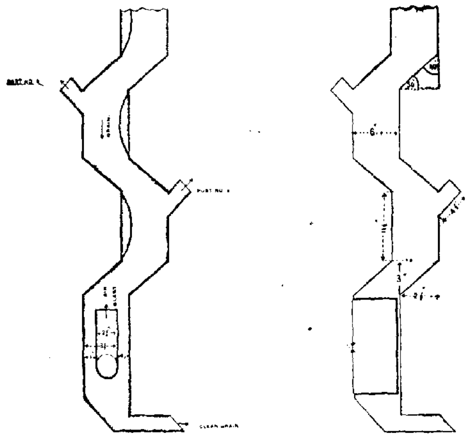


Fig. 12.

of thin sheet iron) with a number of bends, each bend making an angle of 40° with the perpendicular, the object of which is to help neutralize the increasing momentum of the falling grain due to gravitational acceleration. With a tube of sufficient length divided into a sufficient number of steps, we can, in this way, obtain a falling grain of uniform velocity, instead of increasing velocity. If now this grain meets a current of air flowing up the tube, we can by increasing the velocity of the latter ultimately reach a point when its momentum is equal to the momentum of the falling grain. At this point the grain will "*float*" if we go beyond this, and increase the velocity of the air blast, the grain will be driven back up the tube.

The density of air at 30°C. (a fair average temperature for the tropics) is 0·001165 (water=1) and the density of wheat is from 1·353 to 1·390, see Table XXXIX.

TABLE XXXIX.

Specific gravity of wheat, from the Lyallpur market, compared with water at 15·5°C.

Date of analysis	Description of the sample	Date of purchase	Specific gravity compared to water at 15·5°C	REMARKS
10·6·15	Soft	22·2·15	1·360	Average specific gravity for soft wheat is 1·353.
10-6-15	Soft	30·9·14	1·338	
10·6·15	Soft	7·11·14	1·345	
10 6 15	Soft	28 7·14	1·371	
10-6-15	Soft	29 8·14	1·351	
10·6·15	Hard	30·9·14	1·395	Average specific gravity of hard wheat is 1·390.
10·6·15	Hard	23·10·14	1·382	
10-6-15	Hard	7·10·14	1·396	
10·6·15	Hard	2·2·15	1·397	
10·6·15	Hard	1·4·15	1·379	

When the grain starts to fall from the top of the tube we may consider it as a series of particles having no initial velocity ; after falling through a height (h) (in the tube this was 11·5 ") it will have a velocity of :—

$V = \sqrt{2gh}.$ less air friction. It now impinges at an angle of (α) degrees (in this case $\alpha = 40°$) on a sloping surface, from this it will rebound at an angle of θ and will then have a velocity of U where : —

$$U = V\sqrt{\text{Sin}^2\alpha - \xi^2\text{Cos}^2\alpha} \text{ — losses by aerial friction.}$$

for :—

$$U \text{ Sin } \theta = V \text{ Sin } \alpha$$

and the relative velocity along the common normal after impact is ($-\xi$) times the relative velocity before impact.

{(ξ) is the coefficient of restitution or elasticity, in the case of wheat and iron this is not known.}

$$U \cos\theta - 0 = -\xi(-V\cos\alpha - 0)$$

$$\therefore U\cos\theta = \xi V\cos\alpha$$

from which we have

$$U = V\sqrt{\text{Sin}^2\alpha - \xi^2\cos^2\alpha}$$

The impulse of the blow will be

$$m\,V\cos\alpha + m\,U\cos\alpha$$

$$= m\,(I + \xi)\,V\cos\alpha.$$

This represents loss of energy by impact. If the angle θ is small the grain will start to slide down this sloping surface which makes an angle of (β) with the horizontal and we have :—

V_1 = velocity after sliding over a length (l) with a frictional coefficient of (μ) and initial velocity of U.

We then have

$V_1 = U + \sqrt{2gl\,(\sin\beta - \mu\cos\beta} - $losses by aerial friction.

From this point it will fall with increasing velocity over the next straight length of tubing of length h and will strike the next sloping tube with a velocity of V_2 where :

$$V_2^2 = (V_1^2 + 2gh) = \text{all air friction}$$

$$V^2 = \sqrt{V_1^2 + 2gh} - \text{all air friction.}$$

The angle of impact is again the same but the velocity has changed from V_1 to V_2. The mass remains constant so that the impact of the blow is now

$$m\,(1 + \xi)\,V_2 \cos\alpha$$

The coefficients of friction between wheat and iron and between wheat and wood are not known but can be easily determined.[1]

The above equation gives the momentum M of the mass (m) of moving grain at any point (p) in the tube. This is

$$M_p = mV_p$$

The air moving up the tube is made to leave the nozzle which is fairly wide, with such velocity that it is able to float out grain having a density less than 1·35 and a velocity of Vp opposite the bottom part. It must therefore have a momentum equal to mVp in the above equation. Making volume unity, *i.e.*, $(1\text{ cm})^3$, m will be equal to 1·35 (volume × density), so that

$$M_p = 1{\cdot}35V_p$$

At this point the momentum of the air will be

$$V_a m = {\cdot}001165\ V_a$$

That is when equilibrium is established between the uprushing air and the falling grain at the point p the grain will have a velocity of

$$V_a = \frac{1{\cdot}35V_p}{{\cdot}001165}$$

In calculating Va we must make allowances for the expansion of the air from the nozzle to the grain tube (in our experiments the cross sectional area of the grain tube was 36 sq. in., while that of the nozzle was 7·86 sq. in.; which mean that the velocity of the air before leaving the nozzle was $\frac{36}{7{\cdot}8}$ times greater than in the outer grain tube).

Data regarding μ and ξ for wheat, wood, and iron will be available in a subsequent publication.

[1] This friction coefficient is measured by the natural tangent of the angle of slope when the grain just starts to slip. Between wheat and cement it is 0·41667 or an angle of rather more than 22°; between wheat and wood or iron it will be less than this.

Now the greatest resistance the wheat will experience in the lower part of the tube will be from friction with the rising air which will not only float out the lighter material during its passage through the tube but will in addition tend to reduce the velocity of all the falling grain to a smaller and smaller degree as the velocity of the air is reduced by losses from the port holes in its passage up the tube.

In our experiment the air blast was derived from a Root's blower, and could not be varied, we therefore altered the design of the tube, *i.e.*, altered the angle of the bends, and the distance between them, until the dimensions given in the diagram were arrived at, when the grain issuing at the bottom was found to be clean and free from damaged grain or insects.

Immediately below each bend of the tube there is an egress port. At each of these a portion of the uprushing current of air escapes carrying with it such matter as is light enough to "float" in it. As we proceed up the tube the amount of air escaping at these ports will become less and less. The mechanical effect of this is, that at the top of the tube the lighter materials, insects, dust, and detritus are removed. As it travels down the tube it meets a stronger and stronger current of air and the heavier damaged materials are blown out; first dust, then shells, and afterwards the more or less damaged grain.

Over each of these ports a muslin bag was fixed to collect the products blown out, and the following table gives the separation achieved on 10·76 kilograms of damaged grain containing dust, weevils, etc. The numbers of the bags are from the top downwards :—

TABLE XL.

Showing the cleaning effect of the grain separator described in the text.

		Quantity of material collected
Port hole 1	64 grams	0·59%
Port hole 2	102 ,,	0·95%
Port hole 3	46 ,,	0·42%
Port hole 4	72 ,,	0·67%
Port hole 5	89 ,,	0·82%
Port hole 6	87 ,,	0·81%
Port hole 7 (bottom) below the air inlet	10300 ,,	95·73%
	Total ..	99·99%

In Table XL, the products collected at port holes 1 and 2 were entirely dust and insects; at port hole 3 the empty shells of grain and cast skins of the larvæ; at 4 empty grain shells and some much damaged grain and at 5 and 6 a less damaged grain. The grain issuing at 7 was clean and free from

adult insects—it contained a very small proportion of slightly damaged grain which showed that beetles probably *A. undulatus* had just commenced to attack it. The advantages of a separator of this type are obvious, there are no moving parts to get out of order as in the case of sieves, the air blast can if required be regulated though this will not be necessary in a large installation; it will only be necessary to see that the fans feeding a given number of separators are running at their proper calculated speed. This can be controlled by the mechanic in charge of the machinery. Lastly the entire products of separation can be collected and used. The dust, detritus, weevils from port holes 1, 2 and 3 can be steamed to kill the weevil and then sold as a manure. This material is rich in nitrogen and phosphates as may be seen from the following table:—

TABLE XLI.

Analyses of the dust, shells and damaged wheat extracted from weevilled wheat by the air blast separator.

	Dust, detritus, dead weevils, etc., port holes 1, 2, 3	Shells port hole 4	Damaged grain from port hole 5	Damaged grain from port hole 6
Total Nitrogen ...	4·51	2·69	1·61	1·58
Total phosphates as P_2O_5.	1·68	1·43	0·80	0·78
Carbohydrates { Glucose	......	......	2·15	1·85
Carbohydrates { Sucrose	Absent	Absent	Absent	Absent
Starch ...	......	......	58·04	61·77
Protein ...	28·18	16·85	10·0	9·8
Germination value ...	Nil	Nil	3·0	46·0

The damaged grains from ports 4 and 5 might be rolled and pressed into cakes and used as a cattle food and that from 6 might find a sale in the bazaar for the preparation of second grade flour. This utilization of the by-products would help to pay off the running costs and the capital outlay on the plant, for as we shall show later the use of wheat cleaning machinery at Karachi has been so profitable there as to complete the purchase of the plant within two years.

The conditions under which mechanical treatment will have to be applied in an Elevator.

In order to render a process of cleaning wheat satisfactory in India, we must, as far as possible, eliminate the necessity for skilled labour and make the method more or less mechanical. It would not do, for example, if we had to employ skilled scientists to supervise the extraction of the weevils

from the stored grain, or to make periodical inspections, though this latter might possibly be arranged for by the Agricultural Department. This point we have kept well in view in the work of which this memoir is a record, and we have as a result of our entomological studies of the life-histories of the principal insects causing damage, been able to arrive at a working scheme which is to all intents mechanical in its application and can be applied by unskilled labour. From the results given in chapter I, it is clear that if we take an infected sample of wheat and clean it by such a mechanical process as described above, there may still be left on the grain after this has undergone the cleaning process, a number of eggs attached to the epidermis of the wheat. In certain months these will develop and pass through their life-history periods more quickly than in others. If we assume that wheat after this preliminary cleaning is always still infected with eggs which will develop later, then, by allowing the wheat to remain a sufficient time in store for the adult insects to emerge and grow but not to reproduce, and then clean the wheat a second time, we shall be able to completely remove all insects causing damage to the grain. If such wheat is stored in chambers constructed, so that there are no cracks or corners in which the insects can lodge, or to which insects can, in the ordinary course of events, gain access, this double treatment of the grain will completely immunise it against weevil attack. On the basis of the above method, we have drawn up the following table showing the time in days which may be allowed to elapse at various periods of the year between the first and second cleaning.

TABLE XLII.

Table for the use in wheat granaries showing the time which may be allowed to elapse between the first cleaning of the grain on entering the granary and the second treatment to remove insects hatched out from eggs which have escaped the action of the cleaner in the first treatment :—

	Period in days, which must elapse between the first and second cleaning	Period in days beyond which the wheat may not remain in the store without a second cleaning
May	10 days	25 days
June	10 ,,	20 ,,
July	10 ,,	20 ,,
August	10 ,,	20 ,,
September ...	10 ,,	15 ,,
October	15 ,,	30 ,,
November	25 ,,	60 ,,
December	30 ,,	100 ,,
January, February and March ...	30 ,,	100 ,,

Example of the application of the table :—

Wheat is received in an elevator and goes into store after cleaning, say in the beginning of June. The collected wheat should be allowed to remain in the store for not less than 10 days and not more than 20 days when it is again passed through the cleaning machinery and afterwards placed in the permanent store bins, where it will keep for an indefinite period (so far as weevils are concerned).

In the construction of wheat granaries in which this method is to be applied, the temporary store bins should be so constructed as to be self emptying—they should have no sharp corners in which insects or dust can lodge, and the bottom of the bin should slope down to a discharging pipe. The permanent store bins should be of the same shape to admit of easy and complete emptying of the bin when this is necessary.

The separators of whatever types used should be situated preferably in the basement where any leakage of weevils, dust or damaged wheat from the pipes conveying them away from the separators may not fall upon and infect grain stored below.

At the end of March 1915 by the courtesy of the director Mr. J. Muller we inspected a wheat cleaning plant belonging to Messrs. Louis Dreyfus at Kiamari, Karachi. This plant was supplied in 1912 by Werner and Co. of Dresden and consists of two parts (*a*) a barley extracting plant, and (*b*) a dust and dirt cleaning plant. Of the two the barley extractor is by far the greater, there being eight cylinders for barley extraction and only one for the removal of dirt.

The cost of the plant at Karachi was Rs. 67,185-10-5 to which must be added Rs. 636-7-3 for the electric installation added in 1914. The cost of the buildings was Rs. 21,980-1-9 and the working expenses for 1914 were Rs. 27,862-11-1 during which period 1,82,537 *candies* of wheat were passed through the machinery. The working costs were 2 annas 5 pies per candy (1 candy = 656 lb. or 0·29286 ton). The repairs executed in 1914 amounted to Rs. 1,245-4-3 and were chiefly for new belting and spare parts for the machinery, this is a low figure and would certainly increase as the machinery ages. The firm calculates depreciation at the rate of annas 2 per *candy* reckoning a 7 years' purchase, but, as a matter of fact, the amount of wheat treated was such as to repay the firm the cost of the plant within two years. Mr. Muller considers this plant will remain in use for 15 years so it will be seen how profitable a cleaning plant of this kind is to a large exporting firm. Other interesting information given by Mr. Muller was to the effect

that the barley extractor is not as useful to them as the dirt extracting machinery, and his firm were negotiating for a new plant at the time of the outbreak of war, to consist of 4 batteries of dirt cleaners only, with a calculated output of 60 tons per hour costing about 2 lakhs in all (building and machinery), all machinery in the newly designed plant to be electrically driven.

The plant we inspected has a guaranteed outturn of 30 tons per hour, but in actual practice the figure of 25 tons per hour has never been exceeded. This falling off is attributed to insufficient sieve accommodation. An inspection of the machinery working on weevilled wheat showed the majority of the dust and weevils were removed in the dirt extractor. The design however is not satisfactory and this part of the machinery is at the top of the building and many weevils escape after extraction and find their way into the cleaned wheat; we found a good many specimens of *R. dominica* in the bagged clean wheat. The sieves too are in our opinion a feature of wheat cleaning machinery which should be reduced to the greatest possible extent.

We give below some details of Messrs. Louis Dreyfus' wheat cleaning plant at Kiamari, Karachi:—

Ground covered by building	28·75 × 10·75 metres.
Ground covered by Powerhouse	8·35 × 6 metres.
Height of building	12 metres at the highest point, average height of roof 8·5 metres.
Cost of machinery supplied by Werner and Co., of Dresden in 1912, working in 1913	Rs. 67,185-10-5

N. B.—Of this sum about Rs. 20.000 is debitable to the cost of the 50 H. P. Oil Motor.

Cost of electric installation for lighting only ...	Rs.	636-7-3
Cost of buildings	,,	21,980-1-9
Working expenses in 1914 on 182,537 candies ... i. e., R. 0/2/5 per candy.	,,	27,862-11-1
Cost of repairs	,,	1245-4-3

The working expense is divided as follows:—

Wages	2 annas per candy
Oil	$2\frac{1}{2}$ pies ,, ,,
Repairs	$1\frac{1}{2}$ pies ,, ,,
Electric fittings and upkeep	1 pie ,, ,,
	Total 2 annas and 5 pies per candy.

For the purpose of wheat elevators in India it would not be necessary to pass the whole of the grain through machinery to clean it from foreign seeds. Such machinery therefore would have to be in proportion to the amount of

grain requiring such treatment. This can be worked out on the new elevator now in course of erection at Lyallpur, and by examining carefully the average quality of the grain passing through any other centre at which it is proposed to erect similar granaries.

But for the purpose of removing dust all Indian grain must pass through a cleaner at least once (until steam threshers and mechanical winnowers have come into more common use). In order to remove weevils and ensure the grain against damage we have shown that it is necessary to pass the wheat through such machinery twice. The size of the plant for cleaning out dust or weevils will therefore have to be commensurate with the capacity of the elevator to allow of a double cleaning of the wheat during the time it is in store, once at entering and once after storing a sufficient time to allow for the incubation of any eggs escaping the first cleaning processes.

LYALLPUR,

July, 1915.

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